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DEVELOPMENTAL STENOSIS OF THE AQUEDUCT OF SYLVIUS*

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In this paper there are presented the clinical and pathologic findings in 11 cases of developmental, non-neoplastic stenosis of the aqueduct of Sylvius. Of this number, 4 represent obvious malformations, whereas the remaining 7 are due to glial proliferations. The possibility that the latter followed inflammatory lesions is discussed, but the weight of evidence indicates that they, too, are developmental in origin. The histologic findings in a large control group of aqueducts of non-hydrocephalic people are presented for comparison. These reveal a range of variation which has been little appreciated and give further support to the theory that stenosis by glial tissue has a developmental basis.

DEVELOPMENT AND FORM OF THE NORMAL AQUEDUCT

The ventricular system develops from the primitive neural tube. At the three-vesicle stage, the aqueduct is as large as other portions of the neural tube. With further development, there is narrowing of the aqueduct. The ependyma forms the major portion of the wall of the embryonic brain. Usually, this lining is one cell layer in thickness and completely surrounds the cavity. Hochstetter[1] found that during the 6th to 16th week there is a loss of neuro-epithelial cells for a short distance on one or both sides of the aqueduct wall. Turkewitsch[2] further described small diverticuli during the development of the aqueduct and demonstrated a number of small wrinkles in its wall in the inferior and ventral portions. These were designated tegmental colliculi.

The aqueduct which develops as a result of this process is about 15 mm. long and as much as 3 mm. wide in the normal adult. The central part is slightly dilated, and was named by Retzius the ventricle of the

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midbrain.[3] Its shape is said to be triangular with the apex directed ventrally, but this varies at different levels. It is lined by the columnar neuro-epithelium called ependyma. Immediately beneath the ependyma is an acellular zone of glial fibrils, surrounded by a collection of astrocytes designated the subependymal cell plate.

Few authors have commented on the variability in appearance of the lining of the normal aqueduct. Spiller[4] studied 38 unselected midbrains and described many variations in its shape and size. He was surprised to find how small an aqueduct was sufficient to permit flow of cerebrospinal fluid without the production of hydrocephalus. In the present study, sections of the midbrain were prepared from 50 non-hydrocephalic brains which served as a control group for the pathologic cases. All sections were taken perpendicular to the long axis of the brain stem, and microscopic slides from them were stained either by the Nissl technic or with hematoxylin and eosin.

In shape, the aqueduct was sometimes triangular with its base either up or down. Oval, round, diamond, T-shapes, and narrow slits, directed vertically or horizontally, also were found (Fig. 1). In size, there was similarly great variation. As far as we are aware, there have been no studies of the dimensions of the adult aqueduct. It is not known how much narrowing must occur to produce internal hydrocephalus. The smallest aqueduct in the non-hydrocephalic series (Fig. 1, G) measured 0.09 sq. cm. in area in the microscopic section.

Variations in the appearance of the ependyma were striking. At times smooth, it frequently presented scalloped edges which may represent tegmental colliculi (Fig. 1, B and C). The ependymal lining was frequently thicker than one cell layer, especially in the lateral and ventral recesses (Fig. 1, B and F). Small accessory aqueductules were of frequent occurrence at variable short distances from the wall of the main aqueduct (Fig. 1, B, C, and E). These commonly occurred at the tip of a projection of the ependyma into the surrounding glial plate. Relatively larger aqueductules sometimes were seen as projections from the ventral extremities of the main passageway (Fig. 1, C). At times, ependymal cells were present in clusters around the wall of the aqueduct without forming small canals.

Frequently there was a loss of ependyma for short stretches on both sides of the aqueduct (Fig. 1, D). Occasionally this was unilateral. These were suggestive of the denuded zones described in the embryo by Hochstetter.[1] Occasionally there was a protrusion of underlying glia through the denuded zone into the lumen of the aqueduct (Fig. 2).

The subependymal glial plate also presented numerous variations in position and cell density. In some instances, there was no well defined plate, but a continuance of the glia of the brain stem up to the ependymal wall (Fig. 1, G). A well defined glial plate sometimes was situated dorsally, sometimes ventrally, sometimes laterally, and at times surrounded the aqueduct completely. Variations in the cellular density of the plate were common.

Report of Cases

MALFORMATIONS OF THE AQUEDUCT, NON-GLIOGENOUS STENOSIS

Case 1 *

E. I. (M. H. autopsy no. 8683), a man, 53 years old, was hydrocephalic from birth. Although slightly retarded mentally, he lived a useful life and was self-supporting. His chief difficulty at this hospital was recurrent bouts of cardiac insufficiency associated with hypertension. No other significant neurologic signs or symptoms were noted. He died during an episode of heart failure.

At autopsy, the extracranial findings included coronary occlusions with myocardial infarcts, and pulmonary emboli with infarcts. The skull was 75 cm. in circumference. The brain weighed 1350 gm. Both lateral ventricles were enormously dilated and communicated with the subarachnoid space through a defect in the corpus callosum. Through this defect, the fornix and basal ganglia were visible (Fig. 3). The third ventricle was enormously dilated. There were multiple irregularities in size and shape of the flattened cerebral gyri.

In the inferior portion of the aqueduct and in the roof of the upper part of the fourth ventricle, a yellowish mass was encountered which could be traced cephalad into the tectum and which distorted the colliculi. The mass was dorsal to the aqueduct and produced gross distortion and striking reduction of the lumen. Obliteration of the iter was not complete.

The mass in the midbrain was partly encapsulated (Fig. 4). It consisted chiefly of adult fatty tissue, with fibrous septa and well formed arteries, veins, and capillaries. Some of the arteries were sclerotic, and many had periarterial nerve trunks. The latter were both myelinated and unmyelinated and had sheaths of Schwann. Small strands of smooth muscle fibers were present also. The margin between the mass and the adjacent neural parenchyma was composed of fibrous tissue in some areas, fat or vascular tissue in others. There was no evidence of invasion of neural tissue by the mass. Small foci of calcification were present in

* This case has been reported previously by Davison.[5]

the midbrain and in blood vessel walls in one small area immediately adjacent to the lipoid mass. Calcification was not present in the mass itself or in its vessels. The leptomeninges of the tectum were replaced by fat and connective tissue in direct continuity with the main mass.

Comment. The tumor mass in case 1 was not a malignant neoplasm. Since there was enlargement of the head at birth, it is clear that the lesion had its origin in prenatal life. There is no evidence of growth of the mass over many years. The patient lived 53 years, and died from other causes. These observations, as well as the position of the mass dorsal to the aqueduct, support the belief that closure of the neural plate early in embryonic life incarcerated a few mesenchymal cells.

This case demonstrates a compensatory mechanism for block of the ventricular system. Although complete obliteration of the aqueduct was not found, it was reduced in volume. An intolerable mechanical situation was prevented from developing because of a defect in the corpus callosum which united the ventricular system with the subarachnoid space (Fig. 3).

Case 2

S. D. (M. H. autopsy no. 10,996) was a colored female, 45 years of age, whose death was due to disseminated visceral and bony metastases from a carcinoma of the breast. Clinically there was no evidence of intracranial disease.

At autopsy there were no cerebral metastases. Marked internal hydrocephalus was present, involving both lateral ventricles symmetrically. The third ventricle also was dilated. A cyst with a granular lining, measuring 1 cm. in diameter, lay adjacent to the aqueduct of Sylvius (Fig. 5, A). The aqueduct was compressed and displaced but there was no communication between aqueduct and cyst. The lining of the former was of flattened columnar ependymal cells whereas the cellular lining of the cyst varied. In its simplest form it was composed of a high columnar epithelium. In other sites, the epithelium formed loops and tufts possessing vascular cores, containing scattered psammoma bodies, and resembling choroid plexus (Fig. 5, B).

Comment. In case 2 the anatomical findings were not complex. A cyst lined by ependymal cells compressed the aqueduct and displaced it from its normal midline position. The similarity in structure of the cyst lining and of that of the ventricular system indicated that the cyst was a congenital diverticulum or an ectopic nest of ependyma. The secretion of fluid into the cyst caused it to enlarge, compressing and displacing the aqueduct and producing partial block with internal hydrocephalus.

As far as we are aware, this is the only instance of a choroid plexus

in the midbrain of man. Kuhlenbeck[6] stated that such structures were found in the aqueduct of the primitive fish, Petromyzon.

*Case 3**

E. V. P. (M. H. no. 0–383) was a woman, 58 years old. She had had a 3-year history of progressive loss of motor power, and was bedridden for the last year of her life, being admitted to a hospital 3 weeks before death. Neurologic examination revealed a right facial palsy, contractures of the upper and lower limbs, and Babinski signs bilaterally. There was incontinence of urine. She had gross memory defects and confabulated. The Wassermann tests of the blood and spinal fluid were negative; this failed to confirm a reported positive Wassermann test of the spinal fluid elsewhere. Terminally, she developed high fever and became comatose. The clinical diagnosis was presenile dementia and "possible lues."

Autopsy revealed brown atrophy of the heart. In the brain there was atrophy of both frontal lobes as well as marked dilatation of the lateral and 3rd ventricles. The aqueduct was obstructed by venous channels beneath its floor and lateral walls. Similar hemangiomatous masses were seen in the right substantia nigra.

Microscopically, the aqueduct was found to be constricted by thin-walled venous channels which were encountered also in the floor of the 4th ventricle and in the medulla. These vessels did not occupy the lumen of the aqueduct but were situated just beneath the ependyma (Fig. 6). Tongues of vessels and glial tissue bulged into the lumen of the iter.

Comment. The lesion in case 3 was a narrowing of the aqueduct of Sylvius by enlarged and deformed venous channels. This resulted in an internal hydrocephalus. Apparently the malformed venous sinuses gradually increased in size during the life of the patient and created a slowly progressive block of the aqueduct.

Case 4

H. R. (M. H. autopsy no. 6683) was a male, 68 years old, who was admitted to the hospital because of vomiting and semi-coma. He had a history of progressive impairment of general activity, mental lability and torpor, and Parkinson's syndrome for about 14 years. Physical examination was notable in that there was bilateral ptosis and complete external ophthalmoplegia. There was marked aphasia, chiefly motor. Coarse and fine tremors and cogwheel rigidity were observed. There was no muscle weakness. The deep reflexes were equal and active, the right Babinski sign was equivocal. Blood urea nitrogen, blood sugar, blood counts, and blood Wassermann examinations were performed, with normal results. Shortly after entering the hospital the patient went into a shock-like state with signs suggesting myocardial infarction, and died.

Post-mortem examination was limited to the cranial contents and eyes. The floor of the 3rd ventricle protruded as a cystic mass the size of a walnut. The tuber cinereum and the corpus callosum were stretched

* A report of this case was given by Davison and Rosenheck.[7]

and thin. The lateral and 3rd ventricles were greatly dilated and there was atrophy of the corpus striatum. The aqueduct was narrow. Microscopically, it assumed a shallow, Y-shaped form on cross section, with almost complete approximation of the ependymal walls, leaving only traces of the lumen (Fig. 7). A few glial cells were seen within the ependymal ring, forming a miniature internal glial plate. The external glial plate was irregularly formed, being absent at some points about the aqueduct, normal in others, wide and deep at still others. At a few points it formed a double band. In places it was separated from the ependyma by a layer of spongioblasts. An inflammatory cellular reaction was not present. The oculomotor nucleus was intact. There was loss of pigment from the cells of the substantia nigra, as frequently seen in parkinsonism.

Comment. In case 4 as well as in cases 1 and 2, the patient lived for a long time with marked narrowing of the aqueduct of Sylvius. There was no evidence of enlargement of the head, and it is impossible from the clinical data to set the time at which the aqueduct became insufficient. Two alternative explanations for the patient's course and anatomical findings may be advanced. The first is that the deformity and stenosis of the aqueduct was completely asymptomatic because the onset was gradual and because of certain mechanisms that may compensate for narrowing of the iter. Other examples of asymptomatic stenosis are given later in this paper, and mechanisms of compensation for aqueductal stenosis will be discussed in another section. The second possible interpretation is that the lesion did produce signs or symptoms, but that they were masked by those of the parkinsonian syndrome. Evidence of an encephalitis as the cause of the aqueductal obstruction in this case is lacking.

GLIOGENOUS STENOSIS OF THE AQUEDUCT

Case 5 *

B. S. (M. H. no. 0–1024) was a male infant who lived 3½ months. Birth weight was 3 lbs., 14 oz. The child was very weak and had to be dropper fed. He became cyanotic during feedings and had irregular respirations. Progressive bulging of the cranium was noted, particularly in the occipital region. The sutures were widely separated and the fontanelles were wide and tense. Spastic paralysis developed in the left arm, and later in the right arm. There were occasional temperature rises. The spinal fluid contained 2 lymphocytes and one polymorphonuclear leukocyte per cmm., 83 mg. per cent of protein, and 700 mg. per cent of chlorides. Wassermann test of the spinal fluid and a colloidal gold curve were negative. A frontal decompression was done, despite which the child became progressively weaker and died.

At autopsy, claw hands, hammer toes, and contractures of the joints were found. There were small foci of pneumonitis bilaterally. The

* This case is reported through the courtesy of Dr. Henry Brody, New York City.

cranial sutures were separated, and the base of the skull was asymmetric. Examination of the brain revealed few gyri and shallow sulci. There was marked internal hydrocephalus distal to an obstructed aqueduct of Sylvius. A layer of thick, green, purulent exudate was seen in the meninges and on some portions of the ventricular ependyma.

Microscopically, the inflammatory reaction in the meninges and ependyma consisted of polymorphonuclear leukocytes and fibrin. There was disorganization of the architecture of the cerebellum. The aqueduct was markedly deformed and consisted of two clusters of miniature aqueducts of unequal size, the smaller dorsal to the larger (Fig. 8, A). Both were surrounded and the smaller one enveloped by glial cell plates which were connected by a broad but rather sparse band of gliacytes. Many of these were astrocytes. The others were indistinguishable from the ependymal cells that formed the tiny aqueducts. It was particularly noted that the inflammatory reaction in the meninges did not involve these structures.

Comment. There is little doubt that the stenosis of the aqueduct in case 5 was present at birth and was nearly complete. The existence of two aqueductal structures, rather widely separated, suggests that during intra-uterine development the aqueduct was divided into two segments, one dorsal and the other ventral. The congenital lesion characterized by multiple miniature aqueducts in a distorted pattern, with changes in arrangement of the glial cell plate and the individual glia, is emphasized. Congenital deformities of the extremities, asymmetry of the base of the skull and the disorganization of the cellular pattern of the cerebellum serve to suggest that the aqueductal stenosis also was congenital.

Case 6 *

P. B. (M. H. autopsy no. 10475), a colored boy, 7¾ years old, was admitted because of a 1-year history of slow enlargement of the head. His school work had deteriorated in quality. There were brief episodes during which he seemed out of contact with his environment and dropped objects from his hands. An ataxic gait and slowness of speech developed. Headaches appeared, and there was some vomiting. Examination revealed separation of the cranial sutures with a positive Macewen's sign. There were right pyramidal tract signs, and an ataxic gait. Funduscopy and roentgenograms of the skull indicated increased intracranial pressure. Occipital craniotomy failed to demonstrate the expected tumor of the posterior fossa, but internal hydrocephalus was discovered by needling a lateral ventricle. About 9 days later, evidence of meningitis appeared, but no bacterial agent could be identified on multiple spinal fluid cultures. A transfrontal craniotomy performed subsequently also failed to disclose a neoplasm and again there were no findings except hydrocephalus. At a third operation a right choroid plexectomy was done and the course thereafter was rapidly downhill. The total duration of illness was 15 months.

Necropsy revealed pulmonary congestion and edema, with bilateral

* We are grateful to Dr. Leo Davidoff, New York City, for permission to report this case.

bronchopneumonia. There were an accessory rudimentary fissure at the base of the left lung and undescended infantile testes. The head was hydrocephalic, with a circumference of 55 cm. The anterior fontanelle was patent, measuring 2.5 cm. in greatest length. The brain weighed 1160 gm. During its removal, the thin floor of the 3rd ventricle was torn, releasing a large amount of ventricular fluid. There was tremendous internal hydrocephalus. The aqueduct of Sylvius was discolored and occluded.

On microscopic examination the midbrain was found to contain many miniature aqueducts, most of which were distorted, invaginated, and apparently invaded by nodules of large astrocytes. Some parts of the wall were one ependymal cell in thickness, in others the cells were stratified. The aqueducts were arranged in a rough circle with a "handle" directed ventrally (Fig. 8, B). There was an inflammatory reaction in the aqueductal region which consisted of an infiltration by polymorphonuclear leukocytes and mononuclear cells, accompanied by a few small fresh hemorrhages. No other case in this series had such a reaction.

The periaqueductal glial apparatus was of interest. It occurred in two positions: the first was external to the ring of minute aqueducts and was discontinuous about them, but not unusual in outline or cell density; the second or internal glial cell plate was a less well organized mass that occupied the center of the ring of aqueducts (Fig. 8, B). It mingled with small clusters of ependymal cells that comprised the ring. Some of them formed tongues and nodules of astrocytes that invaded or invaginated the small aqueducts. Many of the cells of this internal plate were astrocytes with large nuclei and abundant cytoplasm. Ependymal cells formed small acini in which many grades of differentiation appeared, ranging from clusters of cells to well defined tiny aqueducts. Elsewhere in the internal plate, glial cells were arranged in swirling fascicular bundles accompanied by bands of glial fibers.

Comment. The problem in case 6 was to determine whether the inflammation produced gliogenous stenosis of the aqueduct or followed it. The presence of inflammatory cells is not evidence that the basic process was necessarily infectious. It seems probable that it resulted from the operative procedures and from sterile meningitis. It is important to note that there were 6½ years of apparently normal life. Despite this, the finding of an enlarged head and a patent anterior fontanelle indicated increased intracranial pressure early in life. Thus, the evidence points to a developmental lesion on which an inflammatory process was superimposed late in the course.

Case 7

F. F. (M. H. autopsy no. 3836), a white male, was born with an enlarged head. His life until 13 years of age was marked by some retardation of general mental and motor performance. Walking was not accomplished until 2 years of age, but he learned to walk naturally. He began school at age 6, but did not do well and was put in an ungraded class. At 13 years of age, he began to drag his left foot and held his left arm flexed at the elbow. One year later, paroxysms of frontal and occipital headaches appeared. There was vomiting and slight fever, tremors of the left extremities, and retraction of the head. These continued and he entered the hospital. Examination showed an enlarged, bulging head and left hemiparesis with spasticity. Reflexes were recorded as irregular and unequal. There was a Babinski sign on the left. No sensory changes were noted. The eyes were normal except for bilateral atrophy of the optic nerve, considered to be secondary to papilledema. There was bulbar speech. Some pigmented spots in the skin were diagnosed clinically as von Recklinghausen's disease.

The episodes of headache, fever, and retraction of the head recurred with increasing frequency. He would be apathetic or comatose when they occurred. Kernig's and Brudzinski's signs were obtained during these attacks. Blood counts, urine examinations, and blood sugar and urea determinations gave normal values. Wassermann test of the spinal fluid was negative. Four separate specimens of spinal fluid were taken: two were described as clear, one blood-tinged, and the next, xanthochromic. Cultures were not made. The patient died at 18 years of age during an episode of severe headache and head retraction.

The general autopsy showed nothing of note. The calvarium was thin, with a defect in the right temporal bone. The brain weighed 1630 gm. There was flattening of the cerebral convolutions and a marked cerebellar pressure cone. An enormous hydrocephalic dilatation involved the lateral and 3rd ventricles. The aqueduct was stenotic. A ring of small aqueducts, not unlike those seen in case 6, was found in microscopic preparations through the midbrain (Fig. 8, C). They were well formed and varied in size. An external glial plate was scanty and irregular. The internal plate consisted of clumps and nests of gliacytes which were the source of substantial fasciculi of glial fibers (Fig. 9). They added significantly to the deformity produced by the internal glial plate. Although the fibers originated from astrocytic cells in most instances, they also emanated from ependymal cells. No ganglion cells were seen in the center of the ring. Two minute, abortive aqueducts were present a millimeter or so ventral to the ring. Fibers originating from their lining cells filled their lumina.

Comment. Case 7 illustrates many of the clinical and anatomical features common to the cases of gliogenous stenosis. The history gives evidence that a block of the aqueduct existed since the time of birth. The 18-year life span in this patient is noteworthy. The attacks of headache, fever, meningeal signs, and retraction of the head conceivably reflect episodes of acute increase in intraventricular pressure.

Case 8

C. P. (M. H. autopsy no. 0–391) was a white male, 31 years of age, who was said to have had hot flashes for many years. His present illness began with bouts of severe headache, nausea, and vomiting. These were accompanied at times by restlessness, irritability and violent excitement, by somnolence and confusion on other occasions. One well observed attack was manifested by headache and severe retraction of the head, with severe pain when the head was flexed. There was no Kernig's sign. Papilledema of 2 diopters was noted. There was bilateral ptosis and lateral deviation of the right eye. Pupils were small and irregular, with slight reaction to light and no reaction on accommodation. There was paralysis of convergence and upward gaze. A slight central facial weakness was noted. The gait was staggering and had a broad base. The deep reflexes were hyperactive but there were no abnormal reflexes. Paroxysms continued and the patient died during one of them. The clinical diagnosis was acute internal hydrocephalus due to obstruction of the aqueduct by a neoplasm.

Necropsy examination was limited to the aqueductal region. Internal hydrocephalus was present. The floor of the 3rd ventricle was ruptured while the brain was being removed, releasing a large amount of fluid. The aqueduct of Sylvius was stenotic.

On microscopic examination, severe stenosis of the aqueduct was found at one level, and milder degrees of stenosis at other levels (Fig. 8, D). The almost completely stenotic portion was represented by a thin vertical structure consisting of glial fasciculi arranged longitudinally, a few abortive aqueductules, and two segments of glial plate (Fig. 8, D). The last were most noticeable at the dorsal and ventral extremities of the aqueduct. At the level of maximal stenosis, two minute slits without ependymal lining were seen. Sections from areas of less complete stenosis had the same appearance, except that a slightly larger vertical slit was present in the center of the glial plate. The slit had no ependymal lining but represented aqueduct. Cephalad sections showed the corresponding structure to have a partial ependymal lining. There were mild perivascular round cell collections at the level of maximal stenosis. A cellular inflammatory reaction was absent in other parts of the midbrain and in its meninges.

Comment. This patient (case 8) had the shortest clinical course in the series. Sections of the aqueduct contained multiple aqueductules, deformities of the glial plate, and overproduction of glial fibers, features seen in clearly congenital stenosis. The cellular inflammatory reaction was mild and entirely perivascular. It seems doubtful that it played an important rôle in the stenosis. There was no clinical evidence that this man was hydrocephalic, in contrast with the cases already cited. Nevertheless, this case and those following demonstrate that a congenital or developmental stenosis of the aqueduct may be found in adults with-

out enlargement of the head, who may be well symptomatically for long periods of time.

Case 9

N. K. (M. H. autopsy no. 6209) was 33 years old at the time of death, and had been ill for the last 12 years. The family history was inadequate, but contained accounts of "paresis," mental disease, deaf-mutism, and congenital blindness in several members. The past history of this patient included mention of "epilepsy" from age 7 to 9. He was in essentially good health, however, and was employed as a clerk and insurance broker until about 20 years of age, when his vision began to fail gradually and headaches appeared. For the next 5 years he was observed at a hospital because of papilledema, progressive blindness, and finally pallor of the optic disks. The diagnoses were optic neuritis at first, then multiple sclerosis, and later suprasellar neoplasm. When he was 26 years old an anterior craniotomy was performed, revealing no neoplasm in the pituitary region. A subtemporal decompression was done. Three months after this operation the area of decompression was bulging and the disk margins were blurred. His general condition was worse, whereupon he entered Montefiore Hospital (at 26 years of age).

At that time he presented hypesthesia on the left side of the body, left hemiparesis, and bilateral anosmia. The visual fields were constricted and the blind spots were enlarged. The pupils did not react to light, there was a left central facial weakness, and right 12th nerve weakness. Cerebellar signs were absent. For about 3 years the clinical course was characterized by bouts of vomiting and generalized convulsions, the latter occurring particularly when pressure was exerted on the tense decompression site. Other seizures were spontaneous, being generalized or jacksonian and beginning in the left foot. The spinal fluid pressure was increased; actual values were not recorded. An encephalogram showed dilated lateral ventricles and an enlarged sella turcica. At age 30 he was found to have cerebrospinal rhinorrhea. Headache, vomiting, and convulsions continued. He remained in fair condition until death ensued from acute meningitis. The clinical diagnoses were midbrain tumor, rhinorrhea, and acute meningitis.

Post-mortem examination revealed no notable changes in the thorax and abdomen. The calvarium was unusually thin. There was a fistula between the subarachnoid space and the nasal cavity. The brain was tense and swollen, and the floor of the 3rd ventricle was thin. A purulent exudate, in which Gram-positive diplococci were identified by smear, was present around the optic chiasm. Postoperative destruction of the frontal gyri was seen. Dilatation of the 3rd and lateral ventricles was extensive. The lower part of the aqueduct of Sylvius was not patent.

Microscopic preparations of the aqueduct of Sylvius revealed a ring of small aqueducts arranged in a circle with a ventrally directed stalk (Fig. 10). It was especially noted that the aqueductal region was not involved by the inflammatory process. The aqueducts were well formed and resembled the general pattern seen in cases 6 and 7. The external glial plate was continuous about the distorted aqueduct, and had a normal appearance. The inner glial plate was very cellular, and contained many astrocytes, spongioblasts, and small clumps of ependymal

cells. There was a fascicular arrangement of the glia and corresponding swathes of glial fibers similar to those seen in cases 6, 7, and 8.

Comment. Histologically, case 9 corresponds to the other examples of clearly congenital stenosis of the aqueduct. Clinically, except for a vague history of epilepsy in childhood, there is little to indicate disease of the nervous system until the patient was 20 years of age. Thereafter the progressive course is compatible with aqueductal stenosis. It is difficult to explain how such a congenital lesion may exist during many years of relatively normal life. Rhinorrhea which this patient developed at age 30 could have acted as a decompressive mechanism only if there had been a direct communication with the ventricular system above the aqueduct. In this case it appears to have drained only the subarachnoid space.

Case 10

T. N. (M. H. autopsy no. 10,667), a white female, 67 years old, was known to have had hypertension for 5 years. She had suffered from headaches, dizziness, and scotomas during the last few years of life. She entered Montefiore Hospital with signs and symptoms of coronary insufficiency, ventricular failure, and mild hypertension. Neurologic examination showed bilateral diminution of the knee and ankle jerks and a normal plantar response. The fundi were normal. She expired shortly after admission.

Post-mortem examination revealed generalized arteriosclerosis with particular involvement of the renal and coronary arteries, old and recent myocardial infarcts, and pleural effusion. The brain collapsed when it was removed from the skull. The corpus callosum was thin. The lateral and 3rd ventricles were markedly dilated at the expense of the white matter. The aqueduct was narrow.

A series of 12 sections from the midbrain at different levels was studied. Projection tracings of these sections are shown in Figure 12. The lower portion of the aqueduct was a tall, narrow, single, patent orifice lined by normal ependyma. Caps formed by the normal external glial plate surrounded the dorsal and ventral extremities. Higher in the midbrain the aqueduct was shorter and broke up into a cluster of small canals and cysts, while the glial plates, dorsal and ventral, remained separated and formed distinct segments. The dorsal plate accompanied the dwindling aqueduct upward, while the ventral one appeared for a few sections and then ended abruptly. No lumen was visible at the level of maximal stenosis where the glial plate surrounded a sparse, spongy, irregular matrix of glia, in the center of which was a single vertical row of ependymal cells (Fig. 11). Above this level the aqueduct was devoid of ependyma except for a few small patches in the ventral segment. There was no inflammatory reaction anywhere in the midbrain.

Comment. The integrity of bony structures and the closure of the fontanelles in case 10 indicate that the process of gliogenous stenosis was not fully developed until after the cranial sutures had closed. The absence of inflammation, the distortion of the glial plate, and the deformity of the aqueduct at all levels point to a developmental anomaly as the basis for the stenosis. The absence of ependyma above the level of maximal stenosis is of interest in view of the observation of Hochstetter[1] that in human embryos of 6 to 16 weeks, absence of ependymal cells is a constant finding. This point offers further support for the developmental origin of the stenosis in this case. There was no rupture of the 3rd or lateral ventricles, and no fistulas into the nasal cavity or ear.

Case 11

The patient was a 19-year-old Italian boy (N. H. H. no. 9/35). He was considered normal at birth and his head was not enlarged. Delivery was uncomplicated. The fontanelles closed promptly. His history was not unusual until he entered school. There it was found that he was slower in classwork than other students of the same age. His general health, however, was good. He left school at the sixth grade and did odd jobs, selling newspapers and working as an errand boy in a grocery store. At 17 years of age he had episodes of headaches, nausea, and occasional bouts of vomiting. They would last for 1 to 2 days, which he would spend in bed. There was no history of changes in vision; he did not wear glasses. At 19 years of age an attack developed and he became drowsy. A physician was called and hospitalization advised. Before the patient could be moved, he died in a cyanotic state.

Except for the nervous system the post-mortem examination showed nothing of note. There was generalized flattening of the cerebral convolutions and marked internal hydrocephalus involving the lateral and 3rd ventricles. The aqueduct of Sylvius was completely stenotic. The 4th ventricle was normal in size. The posterior wall of the 3rd ventricle was absent, the cavity communicating directly with a large subarachnoid cyst which lay on the dorsal aspect of the cerebellum (Fig. 13). The quadrigeminal plate was flattened. The pineal body could not be identified, but probably was represented by a greatly flattened disk of tissue lying in the dorsal wall of the arachnoid cyst just beneath the posterior extremity of the corpus callosum.

Microscopically, there was complete obstruction of the aqueduct. The obstruction was produced by astrocytic proliferation. The ependymal lining was interrupted by areas of gliosis, in which there were ependyma-lined aqueductules. The ependymal lining of the aqueduct anteriorly and in the 4th ventricle was largely absent. A few blood vessels about the aqueduct exhibited perivascular lymphocytes. A few fresh petechiae were noted also.

Comment. Case 11 resembles case 7 in the clinical course. Both patients presented mild mental deficiency, attacks of headache, nausea,

and vomiting, with death occurring during such an episode. Both died at about the same age. In case 7, however, there was an enlarged head at birth while in case 11 the first clinical evidence of neurologic disease occurred when the patient entered school. This case provides an example of a third mechanism of compensation for a severe aqueductal obstruction; *i. e.*, rupture or herniation of the posterior wall of the 3rd ventricle with formation of a large cystic reservoir lined by a thin membrane. This reservoir may have provided a semi-permeable membrane for the absorption of ventricular fluid.

Discussion

The age at death of the patients in this series ranged from infancy to late adult life. The reason for this wide span is found in the nature of the individual pathologic processes and their consequences. Minute aqueducts may have acted as accessory pathways for the conduction of cerebrospinal fluid. When the stenosis was complete, it is conceivable that the loose glial stroma was sufficiently porous to act in a similar fashion. The developmental malformation in the aqueduct may have been present at birth, but some patency may have existed which closed in the course of years. In such instances the production of spinal fluid may be depressed, reducing the need for a normal fluid-conducting pathway. In some cases, decompression may be accomplished by rupture of the corpus callosum, the cerebral substance, or the 3rd ventricle. Escape of ventricular fluid through the nose or ear may also aid in decompression. These considerations may be applicable in explaining the long duration of life without notable neurologic symptoms. It will be remembered that 3 of the 11 patients died for reasons unrelated to disease of the nervous system.

All of the patients with gliogenous stenosis had histologically similar lesions. Three types of clinical course were found in patients with this anatomical lesion. In the first group there were no symptoms or signs during life, yet such patients had marked internal hydrocephalus and a stenosis of the aqueduct comparable to that seen in patients with symptoms. In the second group, the patients showed progressive mental deficiency; had intermittent bouts of headache, nausea, and vomiting; occasionally developed oculomotor paralyses; and died in one of many repeated attacks. Lastly, some patients developed sudden block of the ventricular system, resulting in death with increased intracranial pressure.

The evidence for a developmental as opposed to an inflammatory origin of these lesions has been discussed with the individual cases, but

will now be considered as a whole. The congenital origin of the malformations as represented by the first 4 cases is quite clear, and will not be elaborated further. Accounts of lipomas and angiomas in this region are available.[8,9] The group of 7 cases of glial stenosis will now be considered.

The presence of an enlarged head or patent anterior fontanelle in 3 cases indicates that a severe degree of stenosis of the aqueduct was present before the sutures closed. In other cases, the block of the aqueduct was less severe in early infancy, or a compensatory mechanism was present. The study of normal aqueducts has demonstrated that the passage may become quite small without producing hydrocephalus. It is probable that the diameter of the aqueduct is not the only factor concerned in the development of hydrocephalus.

The histologic changes indicative of a developmental deformity of the aqueduct are shown in Figures 8, 9, 10, 11, and 12, and consisted of:

Aqueductules. These were always multiple and appeared in two patterns. The first consisted of a vertical row of small ependyma-lined cavities within the glial plate. Some were satellites of a stenotic aqueduct, others appeared as malformations of the aqueduct itself. The second pattern was a ring of small conduits located between the external and internal glial plates.

Deformity of the Glial Plate. In some instances this was seen as increased numbers of astrocytes forming clumps and strands in abnormal positions. In 3 cases, excessive proliferation of glia resulted in the production of an internal plate which was surrounded by aqueductules as described. In some instances, particularly when an inner plate was present, the deformed glial plate was the source of masses of glial fibers. These appeared both as tangled fasciculi and as large bands. The cells from which they originated were astrocytes, ependymal cells, and spongioblasts.

Pleomorphism of the Cells of the Glial Plate. Notable variability was observed among the gliacytes. The size, shape, nuclear outline, and chromatin content of these cells assumed many forms. In addition to the ependymal cells, recognizable as the lining of small deformed aqueducts, cells of the same appearance were observed both singly and in small clusters. Cells resembling spongioblasts sometimes were present. Their occurrence in mature individuals is additional evidence of malformation.

The possibility of an infectious origin of these lesions was suggested by Dandy and Blackfan.[10] The literature has been reviewed by Globus

and Bergman,[11] who favored a developmental origin. There were no positive serologic tests for syphilis in any of the present cases and histologic changes seen in luetic involvement of the nervous system were not found. A history of meningitis or encephalitis, except terminally, was lacking in all cases. In 3 instances, an acute intracranial inflammation occurred late in the course. In 2 of these 3, the aqueduct was not involved. The only patient with inflammation in the aqueductal region had three operative procedures and an episode of meningitis. In 2 other cases in which death was abrupt, the presence of a few perivascular lymphocytes was regarded as a symptomatic, cellular, inflammatory response. It should be noted that the glial changes around the aqueduct in cases with terminal infections and in the uninfected cases were identical.

Finally, a study of the midbrain in non-hydrocephalic patients disclosed that many of the alterations found in the cases of gliogenous stenosis were present in a smaller degree in "normal" patients. Thus, absence of ependyma, presence of aqueductules, variations in position and cell density of the glial plate, and protrusions of glia into the lumen of the aqueduct, are phenomena which often are associated with non-stenotic aqueducts. The same developmental process, carried further, produces gliogenous stenosis of the aqueduct of Sylvius.

Summary and Conclusions

An account of the development and form of the normal aqueduct is given. The variation in size and shape of the aqueduct, the ependymal lining, and the subependymal cell plate in 50 control cases is demonstrated. Information is lacking as to how small an aqueduct may be without the production of internal hydrocephalus.

Four cases of non-gliogenous malformations of the aqueduct are presented. One, an ependymal cyst containing choroid plexus, is unique in demonstrating such a structure in the midbrain. A case of angiomatous and one of lipomatous malformation in the periaqueductal regions are described. The fourth case represented a congenital coaptation of the walls of the aqueduct.

The clinical and pathologic findings in 7 cases of gliogenous stenosis are reported. Evidence is presented to show that they, also, are developmental in origin. The reasons for the wide range of clinical symptoms and duration of life in stenosis of the iter are discussed. It is demonstrated that some patients with block of the aqueduct and internal hydrocephalus may reach late adult life without detectable clinical symptoms.

Mr. Antol Herskovitz prepared the photomicrographs.

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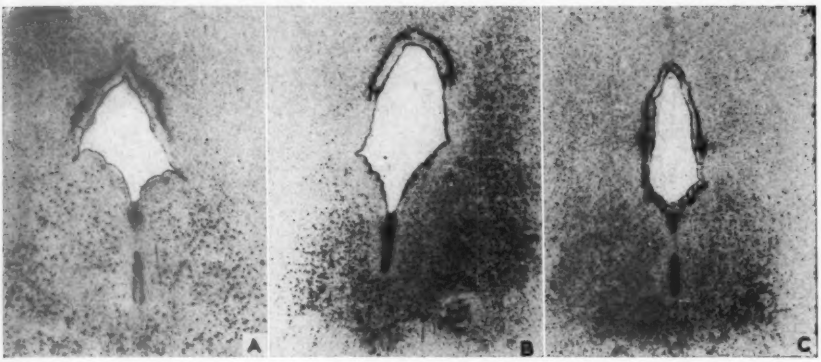
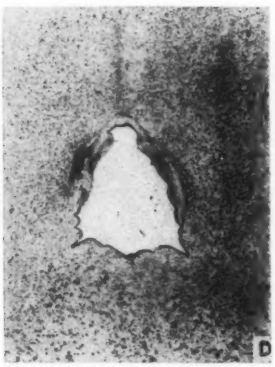
[*Illustrations follow*]

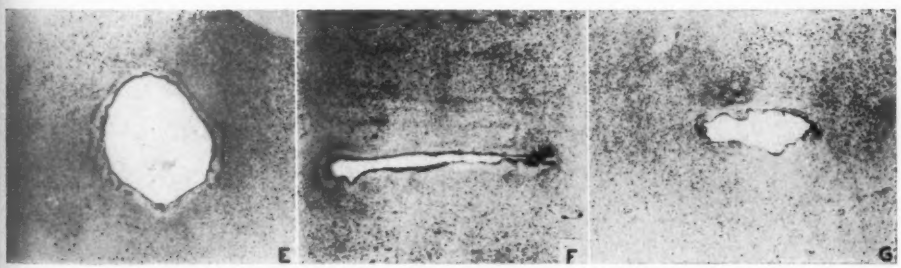
DESCRIPTION OF PLATES

PLATE 99

FIG. 1. Sections through the aqueducts of non-hydrocephalic patients. The variations in structure of the aqueduct, the ependyma, and of the glial plate are described in the text. Nissl's stain. × 35.







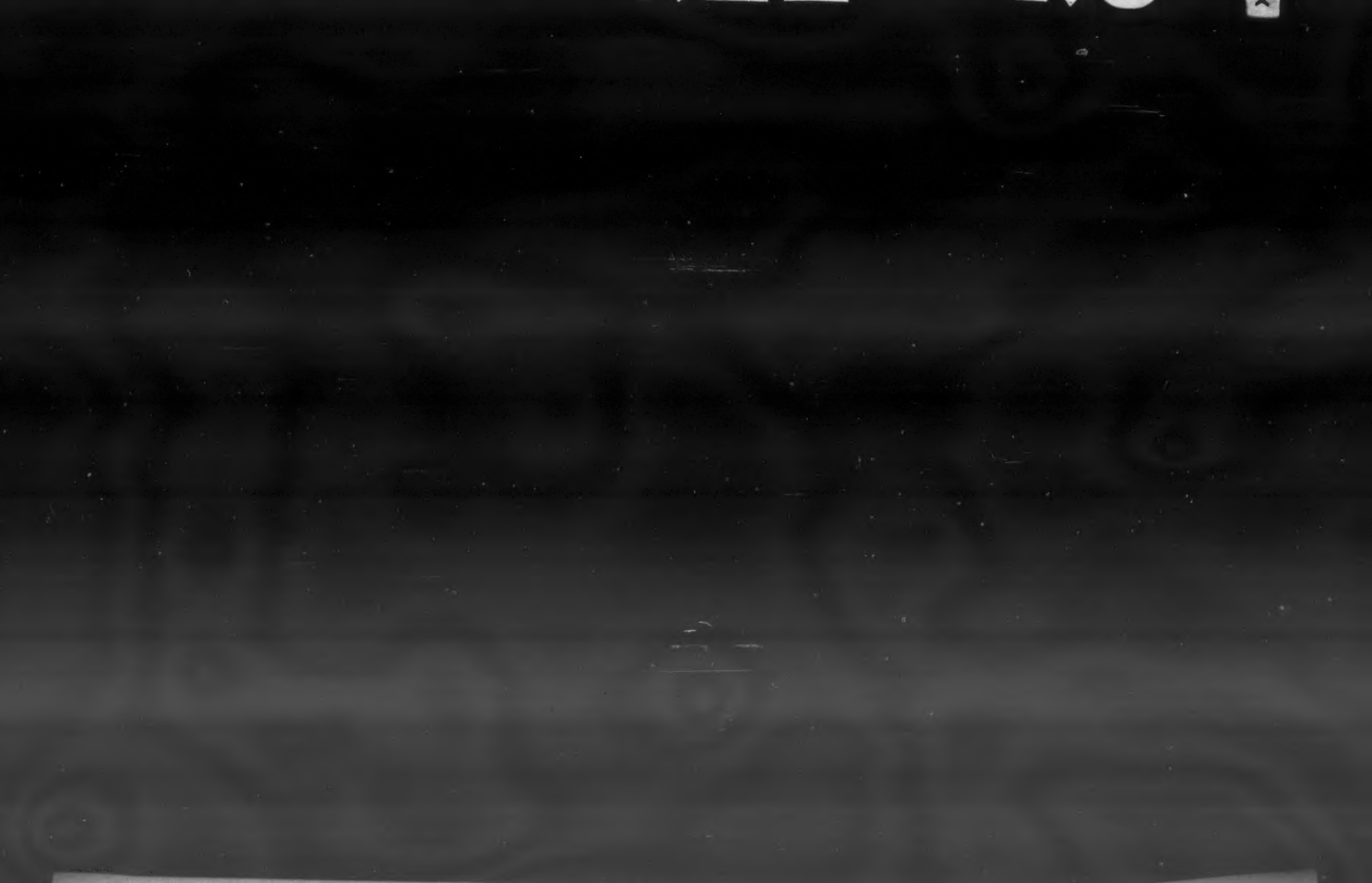
1

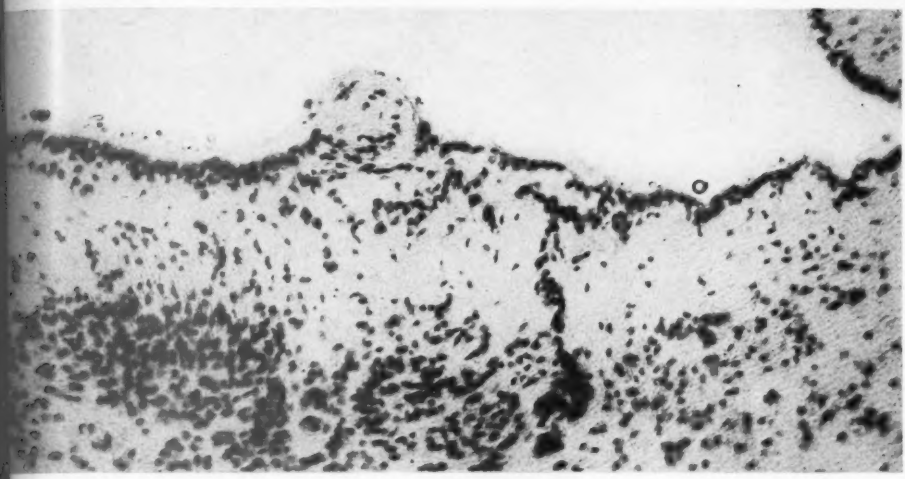
Beckett, Netsky, and Zimmerman Stenosis of the Aqueduct of Sylvius

PLATE 100

FIG. 2. Higher magnification of the wall of the aqueduct in Figure 1, A, showing glial protrusion and absence of ependyma. Nissl's stain. × 155.

FIG. 3. Case 1. The brain, seen from above. A glass rod separates the hemispheres to reveal a marked internal hydrocephalus. The corpus callosum is absent except for remnants. The thin band of tissue passing under the glass rod is the fornix.





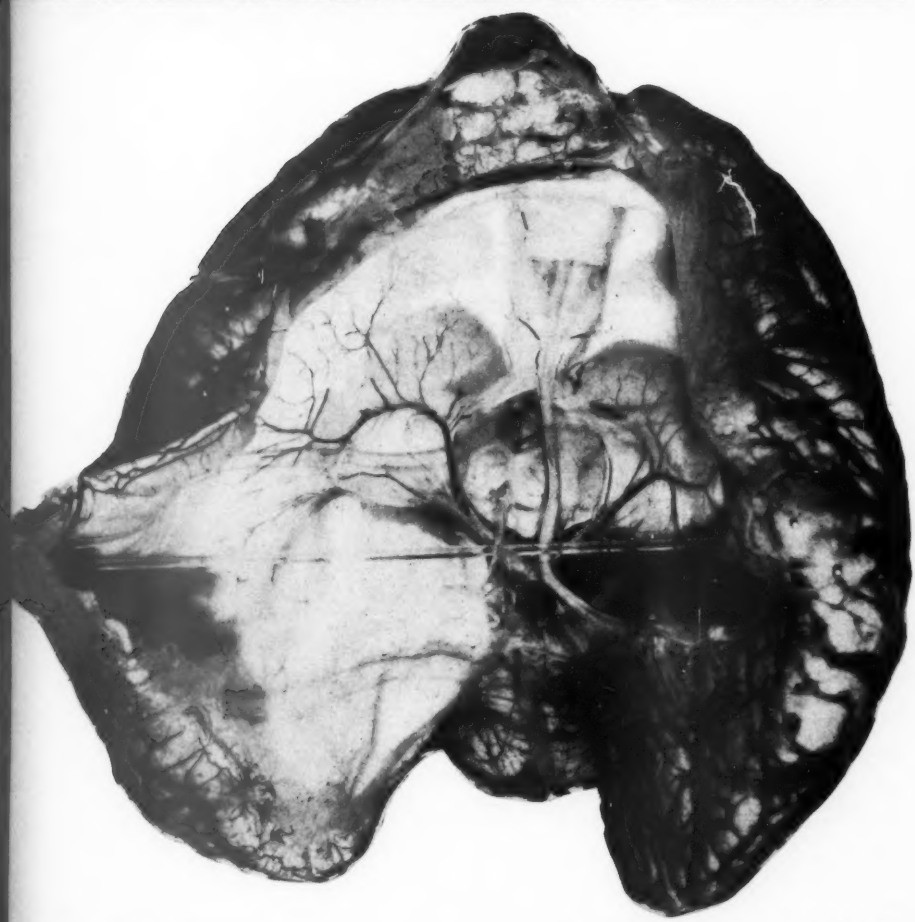


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PLATE 101

FIG. 4. Case 1. The aqueduct is deformed by a fatty mass. There are flecks of calcium scattered through the neural tissue. Van Gieson's stain. × 35.

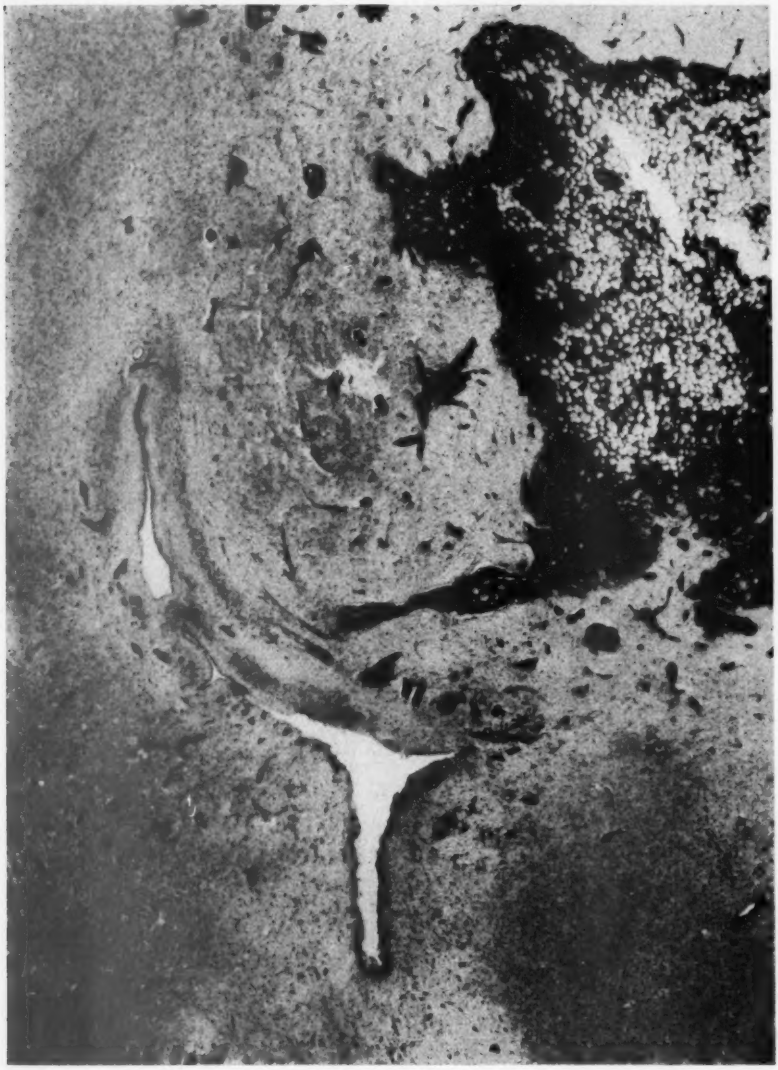

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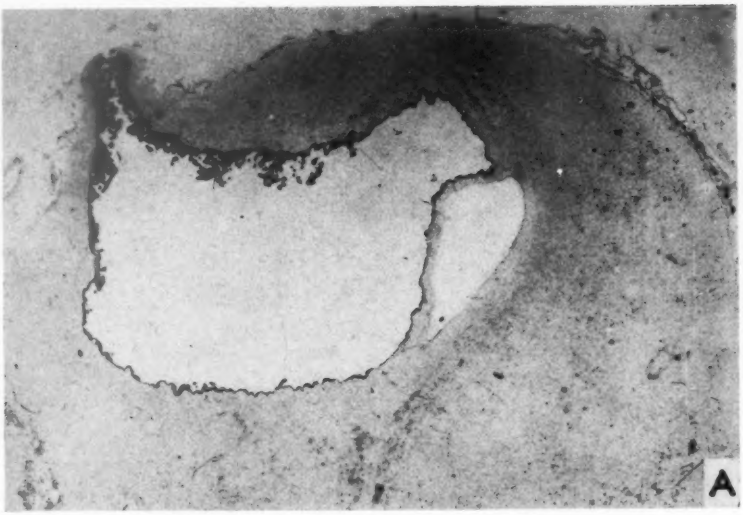
Plate 102

Fig. 5. Case 2. A. The larger orifice is the cyst which has compressed and pushed the aqueduct to one side. Of note is the absence of communication between cyst and aqueduct. Nissl's stain. × 10. B. Higher magnification of the cyst lining, showing choroid plexus. There are a few psammoma bodies. Nissl's stain. × 320.



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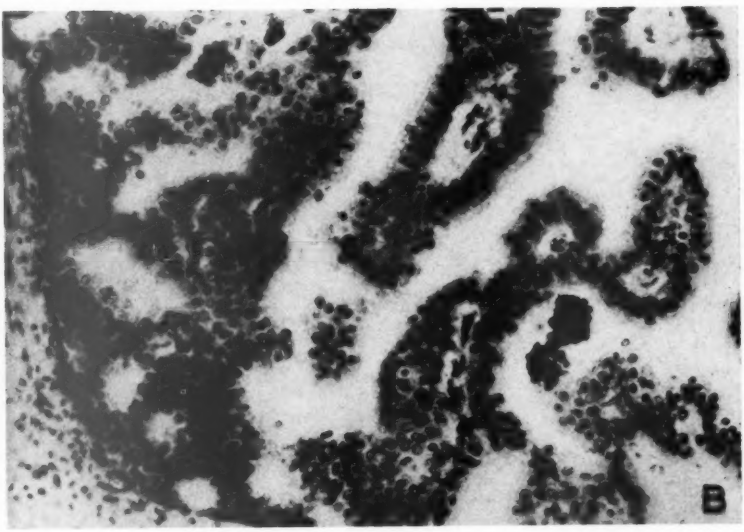

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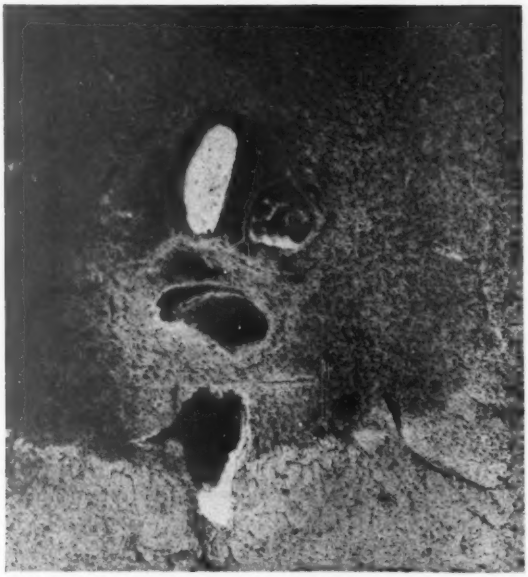
PLATE 103

FIG. 6. Case 3. There are a few malformed vessels near the narrow aqueduct. Nissl's stain. × 35.

FIG. 7. Case 4. The aqueduct is Y-shaped, with the walls closely applied to each other. The central orifice, free of ependyma, may be the result of probing. The upper arm of the Y is the site of a mild gliosis. Nissl's stain. × 35.



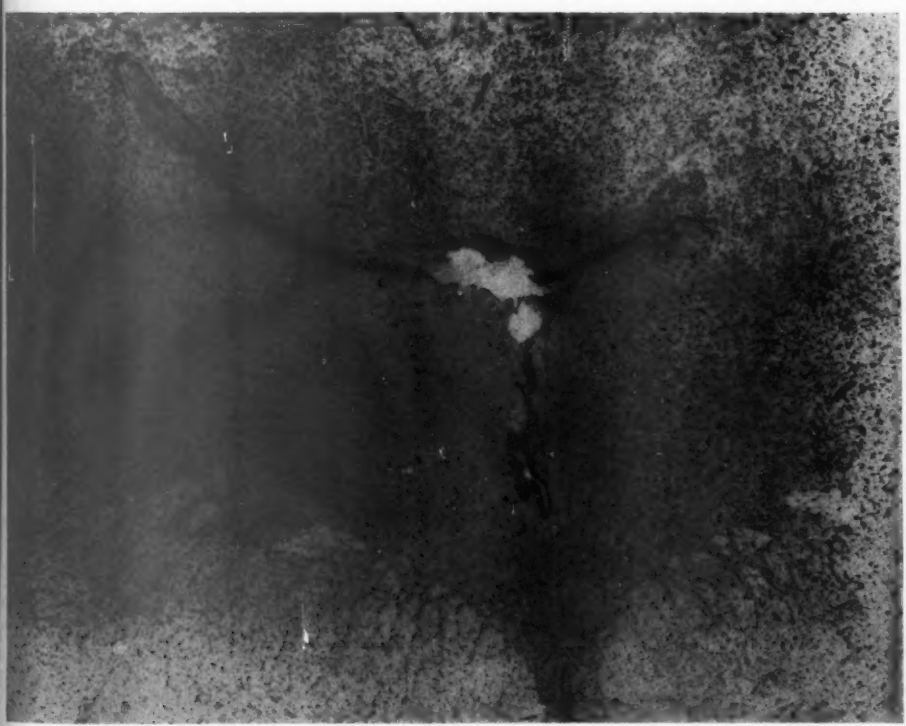


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PLATE 104

FIG. 8. These photomicrographs demonstrate the variations in gliosis and deformity of the aqueduct. A, Case 5. Nissl's stain. × 32. B, Case 6. Nissl's stain. × 32. C, Case 7. Hematoxylin and eosin stain. × 32. D, Case 8. Nissl's stain. × 32.

FIG. 9. Higher magnification of Figure 8, C. The right-hand portion is the external glial plate. One of the aqueductules separates the latter from the internal plate. There are swirls of glial fibers. Hematoxylin and eosin stain. × 293.

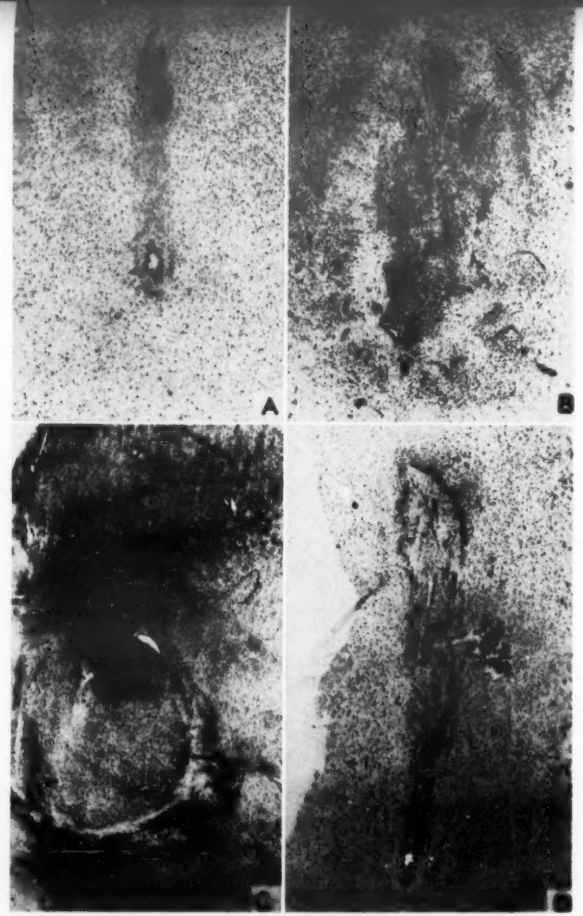


FIG. 8

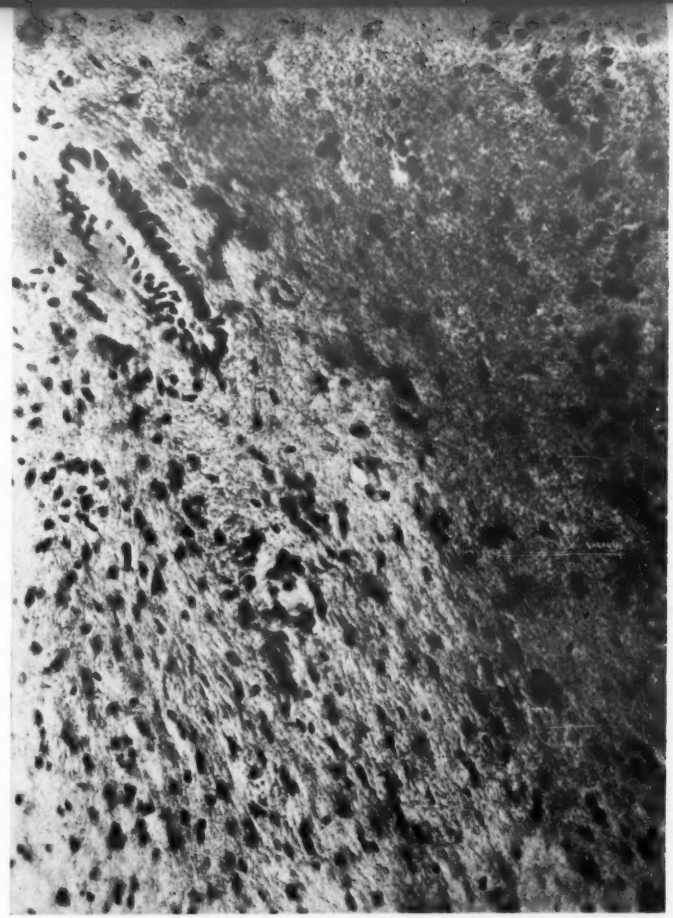


FIG. 9

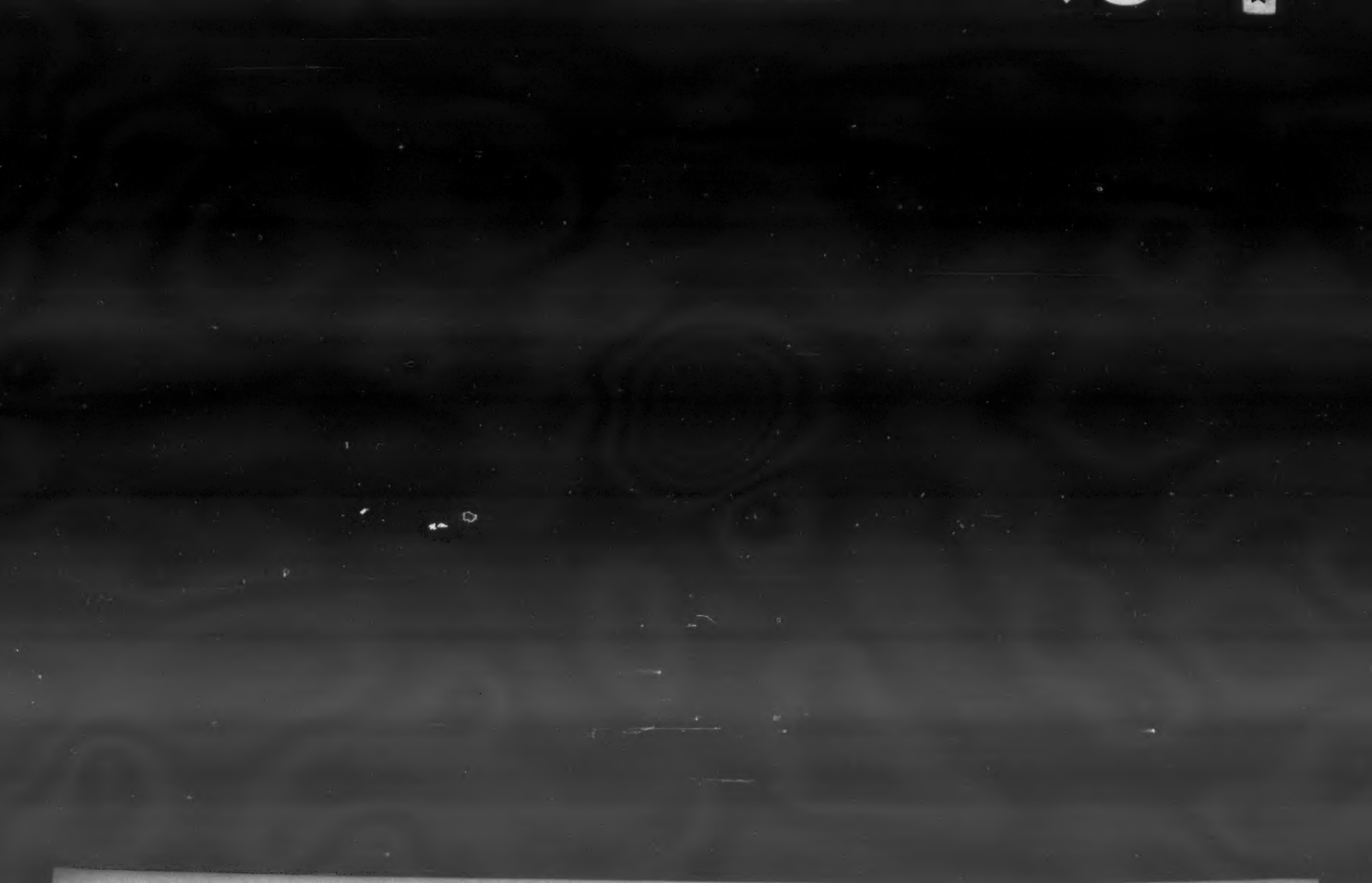
Beckett, Netsky, and Zimmerman

Stenosis of the Aqueduct of Sylvius

PLATE 105

FIG. 10. Case 9. Photomicrograph to demonstrate a ring of aqueductules lying between the internal and external glial plates. Nissl's stain. × 33.

FIG. 11. Case 10. The stenotic aqueduct at level 5. Nissl's stain. × 33.



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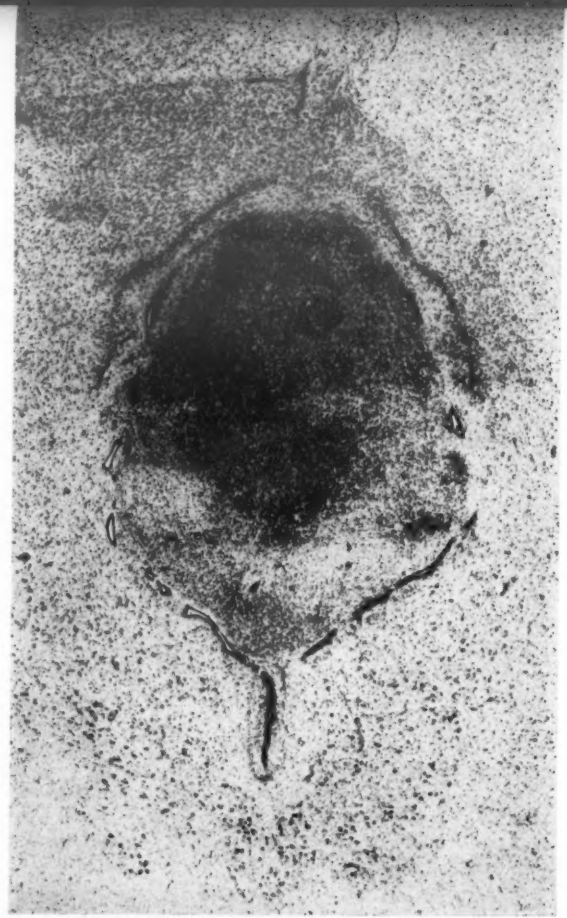

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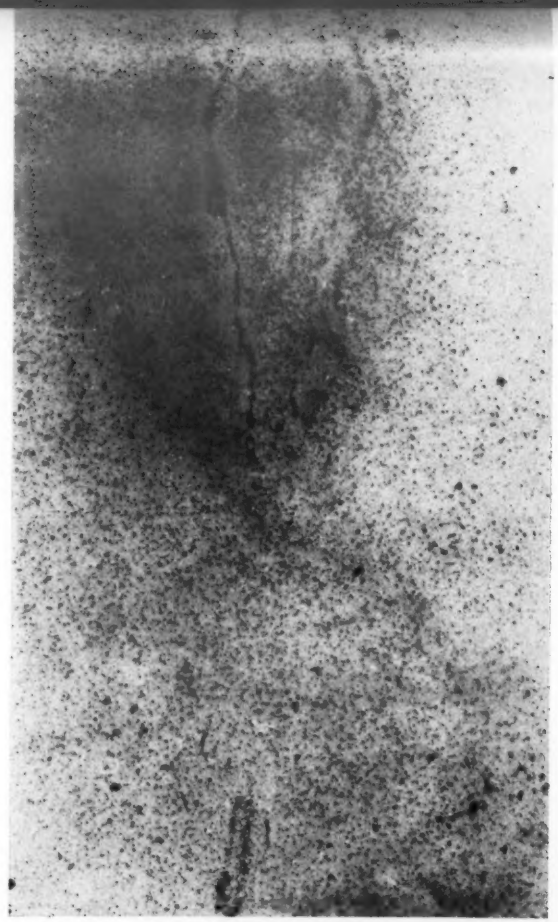
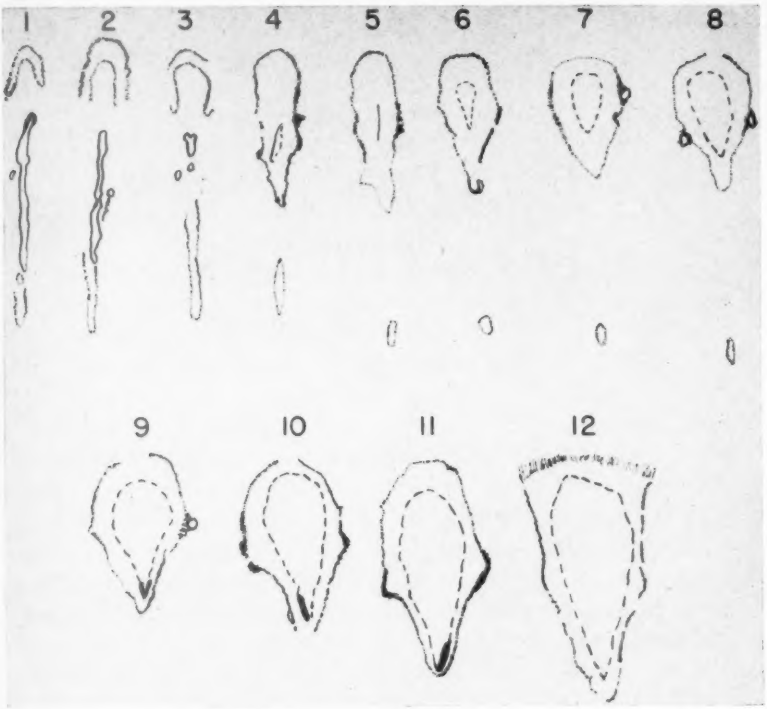


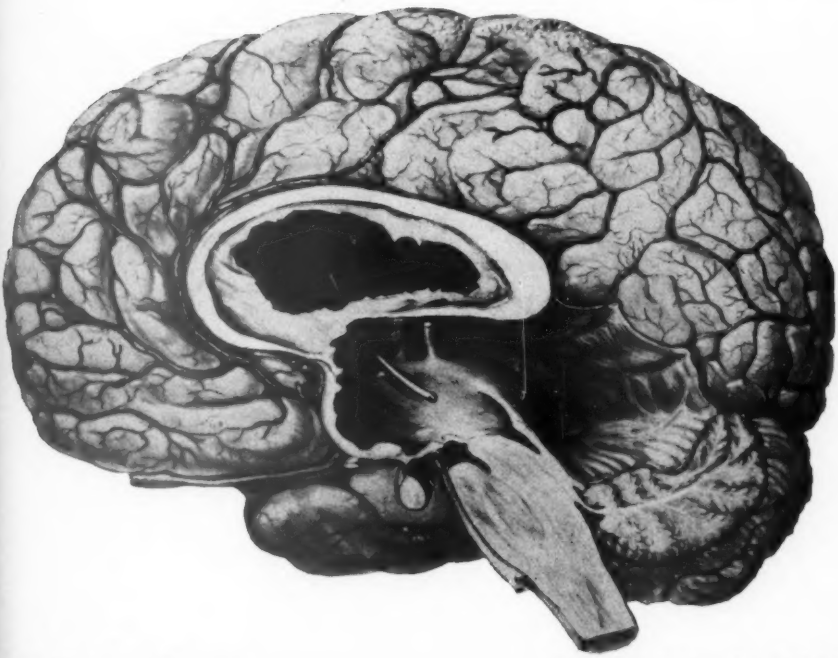
Stenosis of the Aqueduct of Sylvius

PLATE 106

FIG. 12. Case 10. Projection tracings of sections from the midbrain in ascending order. The ependyma-lined aqueduct is shown by the solid lines. Denuded aqueduct is depicted by interrupted lines. Glial plate is represented in fringed outline. At level 5 there is complete stenosis.

FIG. 13. Case 11. Drawing of mid-sagittal section of brain. There is a probe in the interventricular foramen. The third ventricle is enlarged and has herniated posteriorly. The aqueduct is stenotic.





Beckett, Netsky, and Zimmerman Stenosis of the Aqueduct of Sylvius

NECROTIZING PULMONARY ARTERITIS OCCURRING WITH CONGENITAL HEART DISEASE (EISENMENGER COMPLEX)

Report of Case with Necropsy*

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It is the purpose of this paper to report the clinical and pathologic features of a case of congenital heart disease of the Eisenmenger type with necrotizing arteritis (periarteritis nodosa) in which the lesions were limited to the pulmonary arteries. This case is of special interest because of the limitation of the arteritis to the pulmonary arteries.

The various inflammatory changes occurring in arteries which have been generally referred to as "periarteritis nodosa" have been recognized for the past 80 years, although diagnosis of the disseminated disease is infrequently made clinically, principally because of inconstant localization of the arteritis in the body. The exact pathogenesis of these inflammatory changes is unknown, but such factors as bacteria, unidentified toxic substances, viral agents,[1] and certain hypersensitive states[2-10] have been suggested. The concept of sensitivity as a factor has received experimental confirmation from the studies of Rich and Gregory who induced, by experimental sensitization, lesions morphologically identical with periarteritis nodosa, and, coincidentally, cardiac lesions basically similar to human rheumatic carditis.[5,6]

The occurrence of necrotizing arteritis in pulmonary arteries, as reported in the literature, is rare[11-13] and no single instance of limitation of the disease to the pulmonary arteries has been found. We have preferred to use the term necrotizing arteritis as descriptive of the lesions found in this case rather than periarteritis nodosa.

Report of Case‡

A Mexican boy, 11 years of age, entered the Santa Barbara Tuberculosis Sanitarium after a month's illness characterized by fever, cough, and vomiting. Persistent headaches and chilliness had necessitated his being confined to bed. He had been seen by two physicians. The first suggested no diagnosis but prescribed pills which have not been identified; the second suspected pneumonia and prescribed oral penicillin. Because of the boy's increasingly poor condition and night sweats

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‡ This case has been accessioned in the files of the Armed Forces Institute of Pathology, Washington, D.C., as no. 204224.

he was taken to the hospital where he became cyanotic, dyspneic, and disoriented, and died 6 hours after admission. Expectoration of fresh blood was noted shortly before death. The patient was known to have a congenital cardiac lesion which had been recognized at birth. He was usually slightly cyanotic with exertional dyspnea and clubbed terminal phalanges. Examination on admission indicated an acutely ill, poorly developed boy who coughed occasionally. A loud blowing systolic murmur was heard best at the apex.

The erythrocyte count was 4,790,000 and the hemoglobin content 70 per cent. The white blood cell count was 20,550 with a differential distribution of 79 per cent neutrophils and 21 per cent lymphocytes. The urine was not examined. An electrocardiogram disclosed sinus tachycardia and right axis deviation. A single routine anteroposterior roentgenogram of the chest was made. Soft infiltrations 1 to 5 mm. in size were noted in both lung fields, and a diagnosis of miliary tuberculosis was suggested.

NECROPSY REPORT

Gross examination did not include the brain. The skin, kidneys, adrenal glands, pancreas, biliary system, gastro-intestinal and genito-urinary tracts were not grossly abnormal. The significant observations were:

The *heart* weighed 175 gm., which is 53 gm. above the statistical normal. The right ventricle was unusually prominent. The pulmonary conus was shifted to the left and the aorta to the right and anteriorly. A dense shaggy fibrous adhesion, 13 mm. in diameter, bound the right ventricle to the pericardial sac. The opened heart revealed a defect in the membranous part of the interventricular septum, measuring 4 by 11 mm. (Fig. 1). The defect extended from the junction of the anterior and left posterior cusps of the aortic valve to the mid-part of the right posterior cusp. The endocardium over the interventricular septum in the left ventricle was thickened and gray-white. This change was most marked in the region of the defect of the interventricular septum where a triangular pocket, 4 mm. in width and 3 mm. in depth, which opened toward the apex of the left ventricle, was noted in the endocardium of the interventricular septum 7 mm. inferior to the defect (Fig. 1, A). A similar but thicker pocket, 5 mm. in width and 4 mm. in depth, was located in the posterior endocardium of the right ventricle between the right posterior cusp of the aortic valve and the left posterior leaflet of the tricuspid valve; this opened toward the septal defect and the left ventricle (Fig. 1, B) and effectively reduced the area of the former. The margins of the mitral leaflets were irregularly thickened by confluent nodules of firm gray-white connective tissue. The chordae tendinae were short, thickened, and often fused. Foci of fibrous thickening with moderate rolling of the free margin were seen on the two posterior cusps of the aortic valve. There was slight inter-adherence between the two posterior cusps.

The *lungs* together weighed 600 gm., an increase of 200 gm. above the normal mean. They were comparatively uniformly crepitant throughout, although some areas of increased resiliency and of slight nodularity were palpated in the lower lobes. Examination of the cut surface showed single arteries scattered throughout and groups of medium-sized arteries in the lower lobes, which contained shiny black-red clots (Figs. 2 and 3). Smaller arteries 1 to 2 mm. in caliber showed this change most constantly in the more peripheral areas, but arteries to 4 mm. in diameter were involved also, usually as part of a group of altered smaller arteries (Fig. 4).

The difference in the arterial involvement between the upper and lower lobes was striking; it was not related to the density of distribution of the lesions but rather to the tendency for the blocked arteries in the lower lobes to occur in clusters near the hilus. The larger arterial trunks were opened longitudinally and traced to the finer radicles which contained the clots. The intima of the larger vessels was diffusely altered by raised yellow plaques. The clots separated easily from the walls of the smaller arteries, and here the intima was usually not remarkably altered but showed occasional fine granularity. Emphysematous change was noted in all parts of the lungs, but was most marked in the peripheral lobules. The bronchi were not remarkable grossly nor was any alteration in the bronchial arterial system noted. The ductus arteriosus was completely obliterated.

The mediastinal, tracheobronchial, periportal, periaortic, and mesenteric *lymph nodes* were moderately enlarged, with diameters of from 1 to 2 cm.

The *liver* and *spleen* were moderately enlarged, weighing 1150 and 130 gm., respectively. Both organs showed anatomical changes of slight chronic passive congestion.

Culture of the heart's blood showed no growth after 17 days.

Microscopic Examination

Lungs

Sections were taken from grossly involved arteries as well as from the upper and lower lobes in relatively uninvolved parts. The phloxine-methylene blue stain was used routinely, with special stains for the demonstration of collagen, elastic tissue, fibrin, and bacteria. The significant pathologic changes are described under individual headings.

Arteriolosclerosis and Hyperplastic Changes of Muscular Arteries. Small muscular arteries, 2 mm. or less in diameter, showed thickened muscular laminae which were relatively acellular with homogeneous

acidophilic areas, while the arterioles consistently presented thickened medial lamellae and increased periadventitial fibrous tissue. Slight focal subendothelial deposition of loose to compact fibrous tissue with associated endothelial hyperplasia was peculiar to these vessels (Fig. 7). Fibroblasts and loose and compact intercellular ground substance increased the subintimal space and encroached upon the arteriolar lumina. This subintimal arteriolar fibrosis was ubiquitous in the lungs and was demonstrated most commonly as an edematous, uniform thickening with scant cellularity, which caused reduction of the vascular lumina to at least half their potential diameter and often to almost total obliteration (Figs. 5 and 6). Numerous longitudinal sections of the large arterioles disclosed a peculiar tendency for dense, hyalinized deposits of fibrous tissue to occur at the origin of finer ramifications (Fig. 10). No distinction could be made between changes of this type in sections from the upper and lower lobes of the lungs.

Necrotizing Arteritis. A. General Pattern of Pathologic Changes. Single arteries as well as groups of arteries showed lesions. Arterioles and arteries to 4 mm. in diameter were contained in a dense matrix of actively proliferating granulation tissue (Fig. 4). Contiguous structures were involved in the granulomatous process, often to their almost complete extinction. The larger vessels were partially or completely filled with masses of red blood cells enmeshed in loose fibrin. Fibrin masses were not consistently present but, when seen, were in association with fibrinoid degeneration of segments of the arterial wall (Figs. 9, 10, and 11). This fibrinoid change in the larger arteries completely obliterated the endothelial and subendothelial layers and destroyed their muscular laminae, while narrow rings of recent fibrinoid necrosis altered the arterioles in the larger granulomas (Fig. 8). Infiltrations of lymphocytes, plasma cells, and macrophages predominated about the larger arteries, but polymorphonuclear leukocytes were seen most frequently in the segments showing recent fibrinoid change. No eosinophilic leukocytes were noted in association with any of the vascular lesions. Formation of granulomas in the perivascular tissues was characteristic for those muscular arteries altered by fibrinoid change, but was most prominent in the clusters of necrotic arteries seen in the lower lobes and least marked in diseased arteries occurring singly. Arterioles rarely showed primary fibrinoid change in areas separate from the granulomas, although almost all arterioles within a given granuloma were altered.

B. Types of Anatomical Change. Progressive changes were demon-

strated by studies of the numerous small muscular arteries and arterioles.

Acute Stage. The earliest alteration was focal fibrinoid degeneration of cellular and interstitial elements in the subintimal and medial laminae of small arteries and occasional arterioles (Figs. 6, 7, 8, and 9). In muscular arteries, focal edema of the diseased segments in the regions of fibrinoid change separated necrotic collagenous and cellular elements while polymorphonuclear leukocytes infiltrated the adventitia. In the small muscular arteries a tendency for segmental involvement by the necrotizing process was seen and the fibrinoid necrosis often was situated in a segment of an artery adjacent to a smaller ramus (Fig. 10). The few arterioles with lesions encountered separately showed recent necrosis in areas of branching, with infiltrations of polymorphonuclear leukocytes. In the granulomas, arterioles altered by fibrinoid change formed the centers of small islands of polymorphonuclear leukocytes in the midst of widespread mononuclear infiltration.

Stage of Granulation Tissue. The larger arteries containing recent thrombi were examples of early granulomatous formation (Figs. 4, 10, and 11). Necrosis of the intima and of segments of the media was marked, in most instances, and mononuclear cells predominated in the inflammatory exudate. In some arteries partial resolution of necrotic material was seen with proliferation of fibroblasts and capillary buds. Numerous macrophages containing golden brown pigment infiltrated the tissues.

Healed Stage. In several arteries were seen condensations of fibrous tissue elements from which capillaries had partially or completely disappeared. Inflammatory exudate was absent or was seen only as scattered pigment-containing macrophages. The elastic fibers of such arteries were reduced in number or absent, while the remaining elastic fibers often were fragmented and the lamellae disrupted and frayed. Cellular fibrous tissue elements filled the previous vessel lumina and extended through defects in the vascular wall (Figs. 12 and 13). Canalization of the loose connective tissue in the lumina was constant, although such channels were usually of no more than capillary caliber. Occasional arterioles were imbedded in dense condensations of acellular fibrous tissue, often containing granular brown or black pigment.

Chronic Bronchitis and Bronchiolitis. The secondary and tertiary bronchi and the bronchioles frequently were filled with masses of densely packed, granular, homogeneous secretion which gave the stain-

ing reactions of mucin. The epithelium was composed of closely packed tall-columnar cells which were thrown up in attenuated reduplications and large numbers of desquamated epithelial cells were intermingled with the excessive secretion. There was irregular, but constant, thickening of the basement membrane of the bronchial mucosa; the submucosa and bronchial walls were infiltrated with lymphocytes, plasma cells, large mononuclear cells, and occasional polymorphonuclear leukocytes. Muscular hypertrophy was not demonstrated.

The bronchial arteries showed no hyperplasia or necrotizing arteritis.

Heart

Sections were taken from the right and left ventricles to include papillary muscles, the interventricular septum, the right and left coronary arteries to include myocardium, the right ventricle from the area of fibrosis described above, and the right and left auricles. In none of the sections were Aschoff bodies found, nor pathologic changes suggesting acute or healed arteritis.

Lymph Nodes

Sections of mediastinal, mesenteric, periaortic, and splenic lymph nodes disclosed absence of follicles, with active proliferation of large lymphocytes in all parts of the nodes. There was unusual vascularity of the stroma with marked engorgement of blood vessels. The sinusoids were lined by macrophages, particularly in the peripheral parts.

Thymus

Thymic lymphoid tissue was moderately increased by the proliferation of lymphocytic cells.

Microscopic sections of all other parenchymatous organs disclosed no arterial lesions.

ANATOMICAL DIAGNOSES

Partial dextroposition of the aorta with defect of the membranous part of the interventricular septum (Eisenmenger complex); moderate chronic endocarditis of the mitral valve and slight chronic endocarditis of the aortic valve; moderate pulmonary arteriosclerosis and advanced pulmonary arteriolosclerosis; advanced necrotizing pulmonary arteritis of the tertiary radicles and moderate necrotizing pulmonary arteriolitis; moderate chronic bronchitis and bronchiolitis with excess mucus formation; moderate emphysema of the lungs.

DISCUSSION OF PATHOLOGIC FINDINGS

The limitation of the necrotizing arterial disease to the pulmonary arteries and the probable pulmonary hypertension resulting from a

patent interventricular septum of the Eisenmenger type were the points of particular interest in this case. The extensive pulmonary arteriolosclerosis is of special significance because it has the anatomical characteristics of changes seen in the systemic arterioles in essential hypertension. These changes can be readily correlated with recent physiologic studies[14] made upon patients afflicted with congenital cardiac malformations of the Eisenmenger type, in whom direct measurements regularly demonstrated elevated hydrostatic pressure in the right ventricle and pulmonary arteries. These arteriolar changes, with the pulmonary hypertension which they indicate, probably explain the strict localization of the arteritis to this rarely involved arterial system.

Hypertension as a Localizing Factor Limiting the Necrotizing Arteritis to the Pulmonary Arteries

Of approximately 400 cases of periarteritis nodosa reported in the literature,[15] we have found only 48 that occurred in children under the age of 15 years.[16-19] Gruber's review[20] in 1926 noted localization of necrotizing arteritis (periarteritis nodosa) to the arteries of the following organs: kidneys, 74 per cent; heart, 66 per cent; liver, 61 per cent; gastro-intestinal tract and mesentery, 46 and 38 per cent, respectively; musculature, 30 per cent; other special tissues and organs, 1 to 20 per cent. The pulmonary arteries ("lungs") in his study were involved in only 3.7 per cent of the cases. A review by Harris, Lynch, and O'Hare[21] in 1939 omitted all cases not reported in the English language. Their analysis of the necropsy findings of 87 cases gave the following distribution of the arterial lesions: kidneys, 87 per cent; heart, 84 per cent; liver, 71 per cent; spleen, 31 per cent; and lungs, 25 per cent. The difference in the reported incidence of involvement of the lungs in these two series is evident, although Gruber separated the incidence of arteritis in the bronchial arteries (8 per cent) from involvement of the pulmonary arteries ("lungs") (3.7 per cent). Rothstein and Welt,[16] in 1933, reviewed all reported cases of necrotizing arteritis (periarteritis nodosa) in children and listed only 3 cases of involvement of the lung in the 30 cases (33 cases reviewed) with complete necropsy, an incidence of 10 per cent. These reviews clearly indicate that necrotizing processes, while frequent in all systemic arteries, are infrequent or rare in the pulmonary arteries. Necrotizing arteritis is almost always noted in the smaller branches of muscular arteries and the arterioles of the systemic-arterial system.

Since the work of Smith, Zeek, and McGuire[22] and, later, Smith and Zeek[23] has shown an increased incidence of necrotizing arteritis (periarteritis nodosa) in both rats and dogs with experimental hypertension,

it is suggested that the infrequent occurrence of arteritis in the lungs may be partially explained by the paucity of causes for, and the infrequency of, prolonged pulmonary hypertensive states. Dock[24] has emphasized the peculiar susceptibility of the coronary and renal arterioles to alteration by hypertension, an observation that may account for the high incidence of necrotizing arteritis reported in the kidneys and heart. Logue and Mullins[15] made particular note of the occurrence of systemic arterial hypertension at some time during the clinical course in all cases of primary arteritis which they reported.

A recent series of reports have dealt with the physiologic alterations of the pulmonary circulation in various forms of congenital heart disease.[14,25-27] These studies have shown that pulmonary arterial pressures may be elevated inconstantly in a variety of conditions but are increased in every instance of Eisenmenger's complex which could be diagnosed conclusively on clinical grounds.[14] An elevation of pulmonary hydrostatic pressure is postulated by us in the case presented here. The heart showed a single primary anatomical lesion, subjecting the normally isolated pulmonary circulation to systemic hydrostatic pressures through a septal defect with a cross-sectional area which at least equaled that of the aorta. The right ventricular hypertrophy is therefore considered a dynamic response to increased right ventricular hydrostatic pressure.

Pulmonary hydrostatic pressures usually are assumed to be one-fourth of those in the systemic circulation, and by actual measurement systolic pressures in the pulmonary system are 22 to 30 mm. of Hg with a mean pressure of 15 mm. of Hg in normal adults, or 20 to 25 per cent of the systemic systolic pressure.[28] The pulmonary artery, although essentially an elastic artery similar to the aorta, is not naturally constituted to carry internal pressures equal to those in the aorta. Blood normally passes through the lung at low pressures, and the complex, graduated series of muscular arteries and plentiful arterioles found in the systemic arterial system is not provided to reduce pulmonary arterial pressures to capillary levels.[29] The pulmonary arteries are anatomically end arteries, and functionally the necessary pressure gradients are small and to some extent are regulated by changes in intrathoracic pressure during respiration. Therefore, elevations of pulmonary pressure may demand profound arteriolar changes.

The lungs function physiologically as a concentrated capillary bed in close contact with the air, and changing pulmonary minute volumes are compensated by the number of alveolar capillaries patent or collapsed. Thus, with congestive heart failure and mitral stenosis, the

amount of outgoing pulmonary blood is decreased and the alveolar capillaries become engorged, dilated, and show thickening of their basement membranes,[30] a reactive change which was absent in this case. Direct measurements[28] of pressures in the right ventricle have demonstrated that prolonged congestive heart failure may raise pulmonary arterial pressures and confirm this conclusion which was previously reached by Parker and Weiss[30] from the study of the changes in alveolar capillaries and pulmonary arterioles in cases of mitral stenosis. In uncomplicated cases of mitral stenosis, measured pressures in the right ventricle are from 30 to 40 per cent of systemic arterial pressures but may rise to 50 per cent of the systemic arterial pressure with cardiac incompetence.[28] It should be particularly noted that, although the right ventricular systolic pressures cited are but 40 to 50 per cent of the systemic pressures, they represent at least 100 per cent increase in the *pulmonary* hydrostatic pressure. The pulmonary arterial pressures recorded by Bing, Vandam, and Gray[14] in Eisenmenger's complex show systolic, diastolic, and mean hydrostatic pressures of 90 to 95 per cent of the comparable systemic pressures which, if mean values only are taken, represent an increase of 400 to 600 per cent (*i.e.*, 15 mm. for the normal vs. 65, 80, 98, and 100 mm. of Hg). Certainly, changes of this order are not encountered in systemic arterial disease in which systolic pressures are seldom increased more than 100 per cent, and a diastolic increase of 30 to 40 per cent is exceptional. That the marked hyperplastic arteriolar change which was observed in all parts of the lungs in this case was due to marked pulmonary hypertension, has, therefore, a sound physiologic basis; and the gross arteriosclerosis of the larger pulmonary arteries, a change not observed in the systemic arteries, was additional anatomical evidence of such hypertension.

In the cases of mitral stenosis reported by Parker and Weiss,[30] the limitation of the anatomical changes to the lower two-thirds of the lungs in orthopneic patients may be accounted for by the effect of the postural gradient in the pulmonary circulation, recently emphasized by Dock.[24] In our case the uniformity of the arteriolar changes in all lobes of the lungs in the absence of significant heart failure and of changes in the capillary basement membrane (as described by Parker and Weiss in mitral stenosis) indicates that the pulmonary hypertension here is secondary to the interventricular septal defect and is appreciably higher than in cases of mitral stenosis.

The primary lesions of necrotizing arteritis appear to be most common in the smaller muscular arteries. The arterioles are, in general, spared, except in the areas of granulomatous formation; arteriolitis in

this circumstance has the aspects of secondary involvement. These observations are of interest since the physiologic absorption of abnormal hemodynamic pressures by the arterial system would be in the muscular arteries which are expansile and elastic rather than in the arterioles which control only the volume of blood flow.

The arteriolar hyperplasia is of the same nature as that which occurs in the systemic arterial system in response to hypertension. Cases of Eisenmenger's complex have not been described in which lesions of this type are mentioned.[14] The widespread pulmonary arteriolosclerosis could be considered as the anatomical expression of a mechanism by which the elevated pulmonary pressures noted in cases of Eisenmenger's complex are maintained.

The subendocardial mural and mitral and aortic valvular fibrosis is believed to be the result of an abnormal turbulence of the ventricular blood stream induced by the interventricular septal defect. Such anatomical changes have been observed frequently in congenital heart disease and in a wide variety of valvular lesions. Healed rheumatic endocarditis as a cause for the fibrosis of the endocardium and of the valves cannot, however, be definitely excluded.

Summary and Conclusions

In a case of congenital heart disease (Eisenmenger complex), necrotizing arteritis limited to the pulmonary arteries was found. Arteriosclerosis of the pulmonary arteries and advanced arteriolosclerosis of the pulmonary arterioles in all lobes of the lungs, which were not seen in the systemic arteries and arterioles, are regarded as anatomical evidence for pulmonary hypertension. It is suggested that pulmonary hypertension was the result of the congenital cardiac lesion and that this was the important factor limiting the arteritis to the pulmonary arteries.

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DESCRIPTION OF PLATES

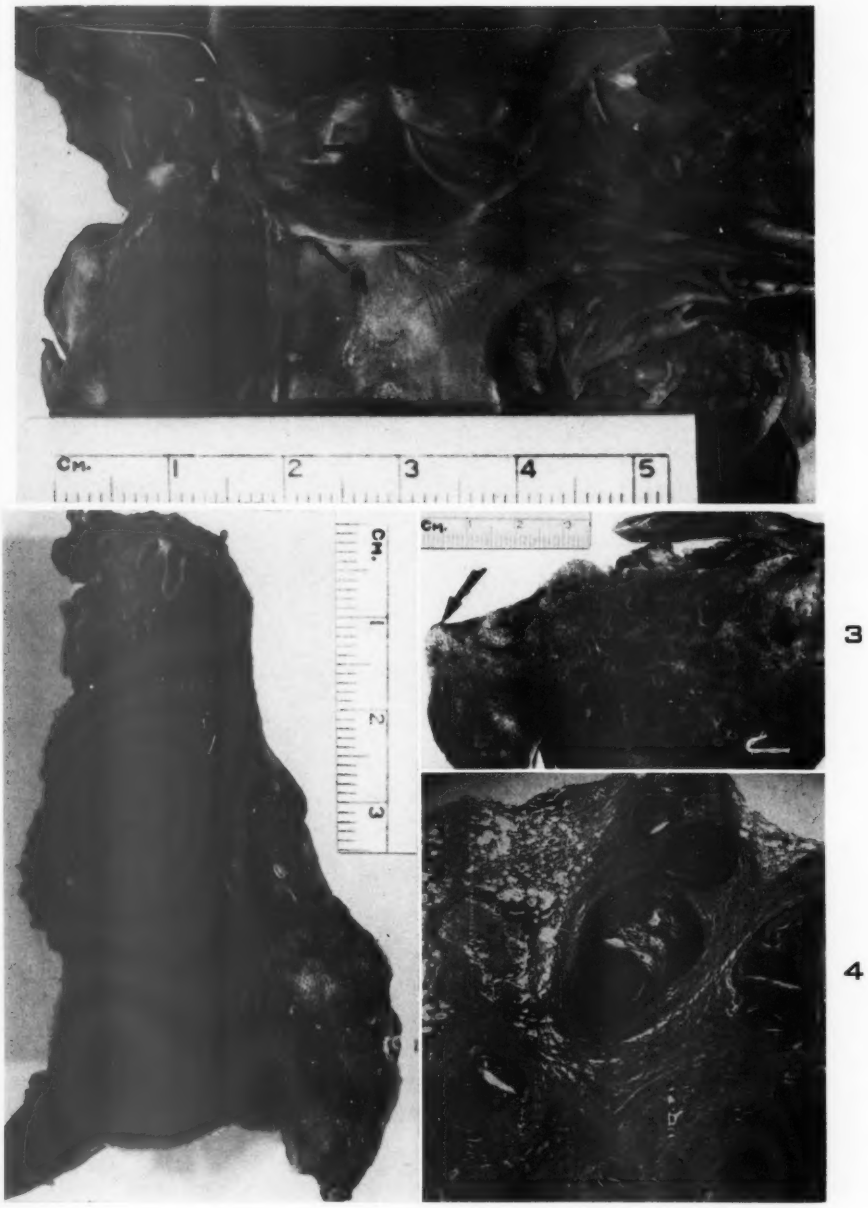
PLATE 107

FIG. 1. The heart is opened to show the chamber of the left ventricle. A triangular defect is seen in the membranous part of the interventricular septum beneath the aortic valve. A. The arrow indicates thickened endocardium and a systolic pocket in the left ventricle. B. Systolic pocket of endocardium in right ventricle, showing also fused and shortened chordae tendineae and nodulation on the ventricular surface of the mitral valve and focal fibrous thickening of the posterior cusps of the aortic valve with inter-adherence.

FIG. 2. A portion of the lower lobe of the right lung showing groups of medium and small arteries filled with black-red thrombus and surrounded by indurated hemorrhagic tissue. The alveolar spaces are visible grossly, indicating emphysema. The extent of arterial change may be compared with Figure 3.

FIG. 3. A section from the upper and middle lobes of the right lung with the apex indicated by the arrow. Scattered arteries contain thrombi. There is nearly complete absence of the arterial lesions in the upper half of the upper lobe and complete absence of grossly visible lesions in the apical part. The distribution and degree of arterial involvement may be compared with that in Figure 2.

FIG. 4. A group of thrombosed arteries surrounded by a granulomatous matrix, from the lower lobe of the right lung. The thrombosis is without evident organization. Phloxine and methylene blue stain. × 25.



Old and Russell Pulmonary Arteritis with Congenital Heart Disease

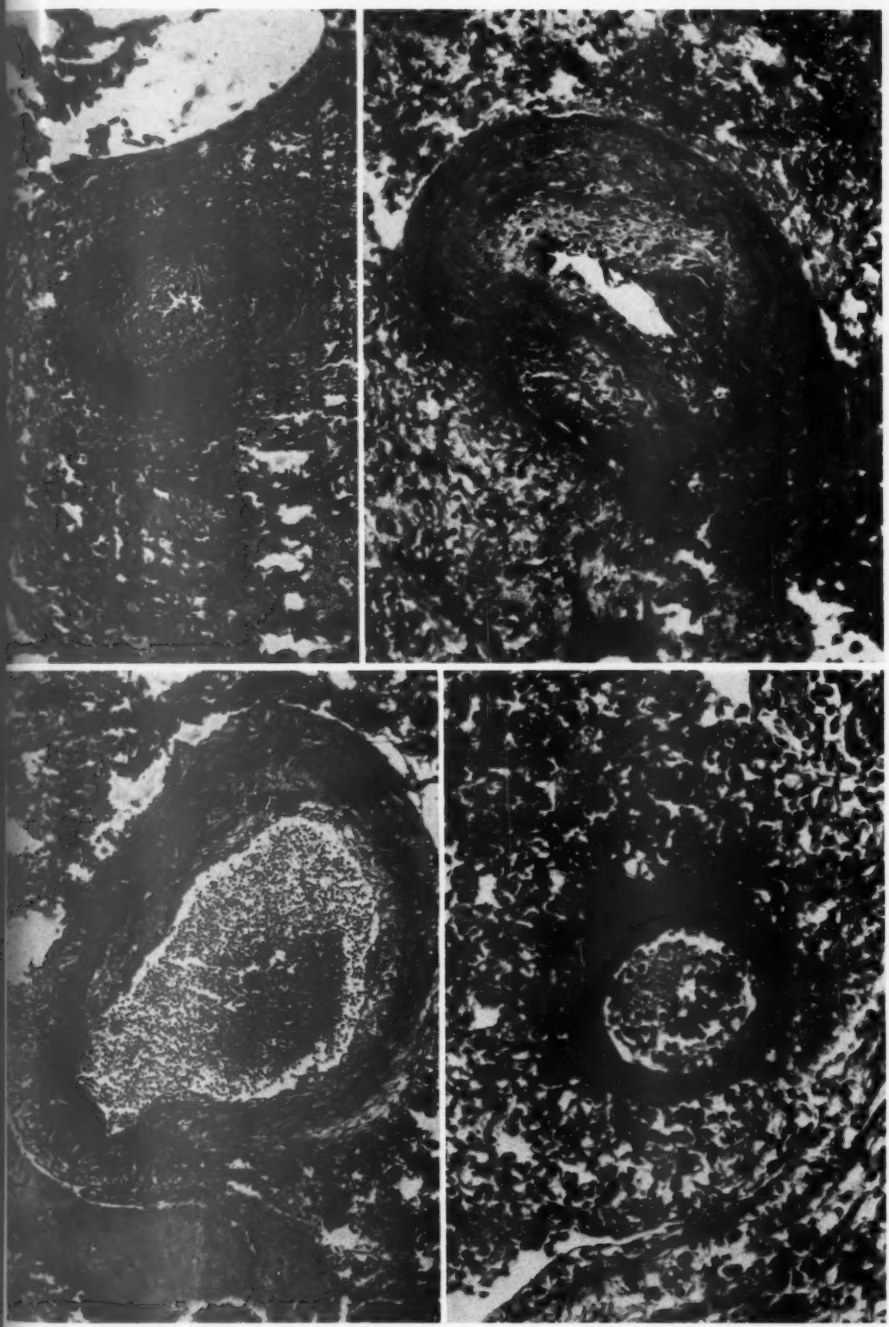
PLATE 108

FIG. 5. Pulmonary arteriole from the lower lobe of the lung showing deposition of subendothelial fibrous tissue with marked narrowing of the lumen. Phloxine and methylene blue stain. × 350.

FIG. 6. Pulmonary arteriole showing subintimal deposition of fibrous tissue and hyalinization of the media in the upper half of the arteriole. Focal fibrinoid necrosis of intima, media, and adventitial tissues is seen in the lower part of the arteriole on the right. Phloxine and methylene blue stain. × 375.

FIG. 7. Small muscular pulmonary artery with subintimal hyperplasia of fibrous tissue clearly demarcated by the internal elastic lamina. Segmental fibrinoid necrosis of the intima and media with edema and infiltration by polymorphonuclear leukocytes is seen in the lower left side of the artery. Phloxine and methylene blue stain. × 100.

FIG. 8. Complete fibrinoid necrosis of a pulmonary arteriole. The adventitial tissues are infiltrated with polymorphonuclear leukocytes and macrophages. Phloxine and methylene blue stain. × 350.



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Old and Russell Pulmonary Arteritis with Congenital Heart Disease

PLATE 109

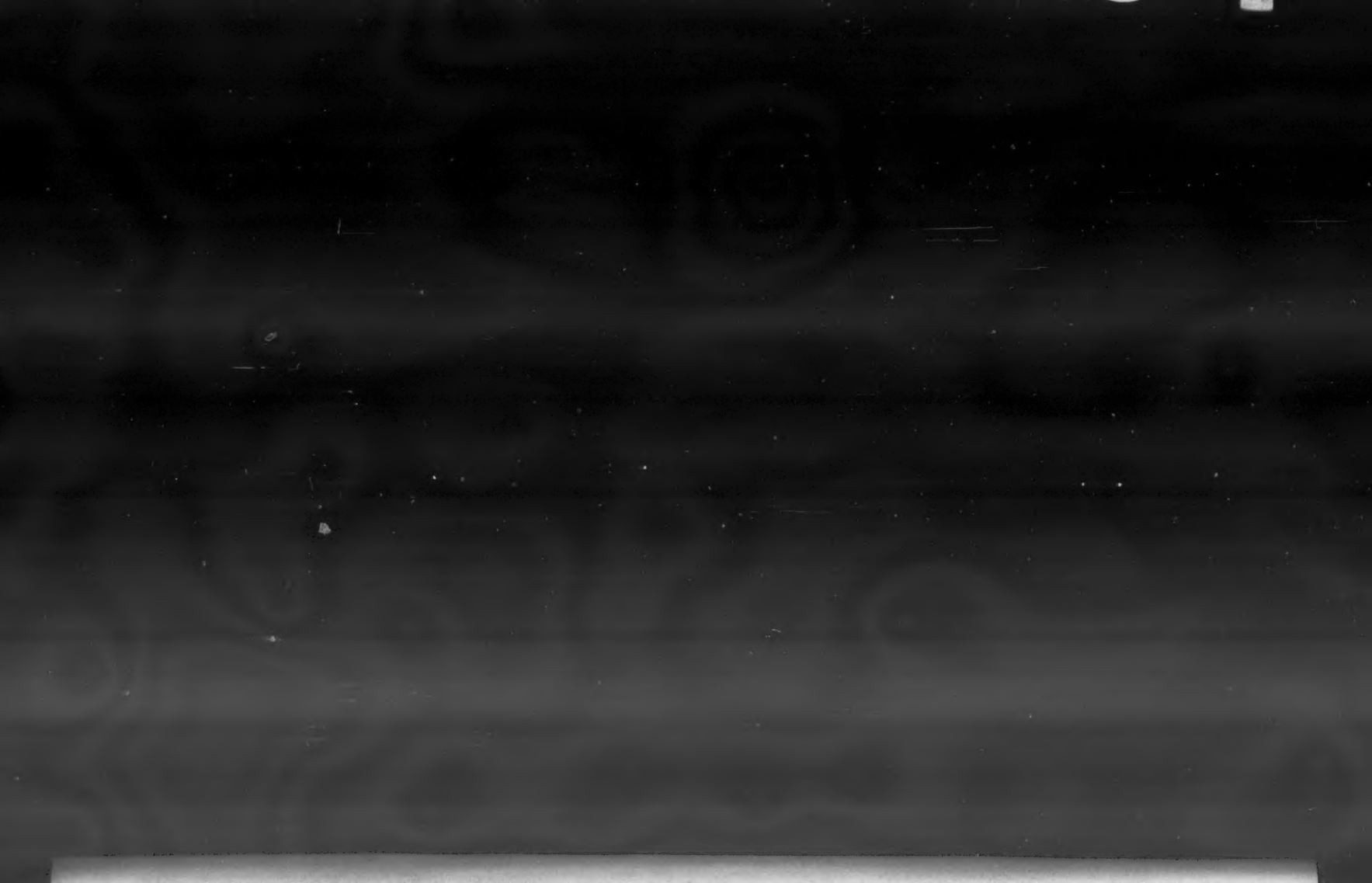
FIG. 9. A small muscular pulmonary artery containing a thrombus and showing necrosis of the entire wall with intensive fibrinoid staining. The necrotic arterial wall and the adventitial tissues are infiltrated with polymorphonuclear leukocytes. Weigert's stain for fibrin and bacteria. × 250.

FIG. 10. Tangential section of small pulmonary artery showing segmental fibrinoid necrosis. Of note is the proximity of a small hyalinized arterial ramus to the necrotic part. A zone of early granuloma formation surrounds the artery. Phloxine and methylene blue stain. × 175.

FIG. 11. Small muscular pulmonary artery showing segmental necrotizing arteritis and a broad zone of granulomatous change. Of note is the more advanced stage of granuloma formation, as compared to Figure 10. Phloxine and methylene blue stain. × 150.

FIG. 12. Two partially recanalized pulmonary arterioles showing condensation of periarterial granulomatous elements. Destruction and fragmentation of the elastic fibers may be seen in the upper arteriole, with nearly complete loss of fibers on the lower side. Weigert's elastic tissue stain. × 225.

FIG. 13. Partially recanalized pulmonary arteriole showing complete destruction of elastic fibers in the upper right segment of the vessel wall. Weigert's elastic tissue stain. × 250.



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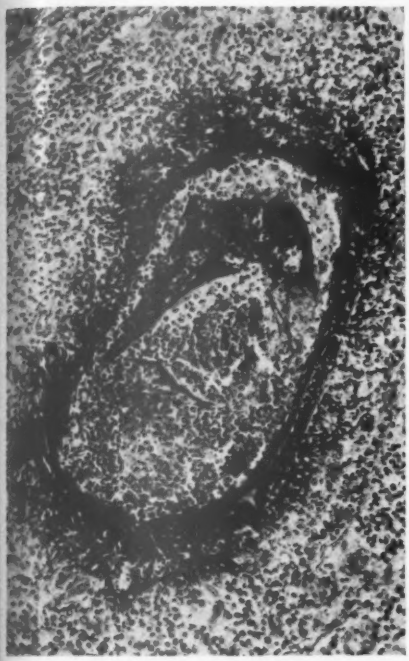

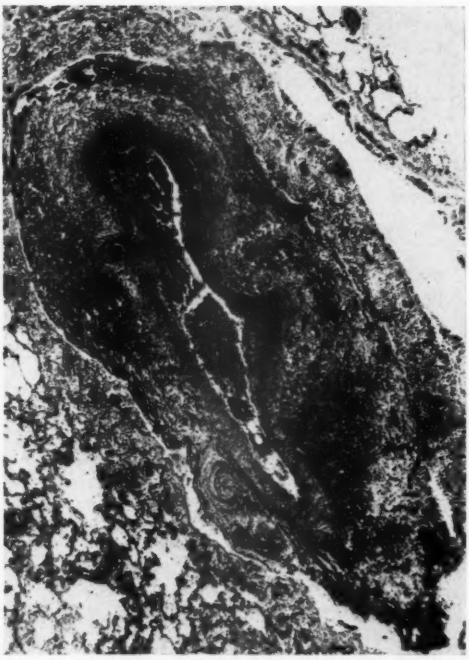

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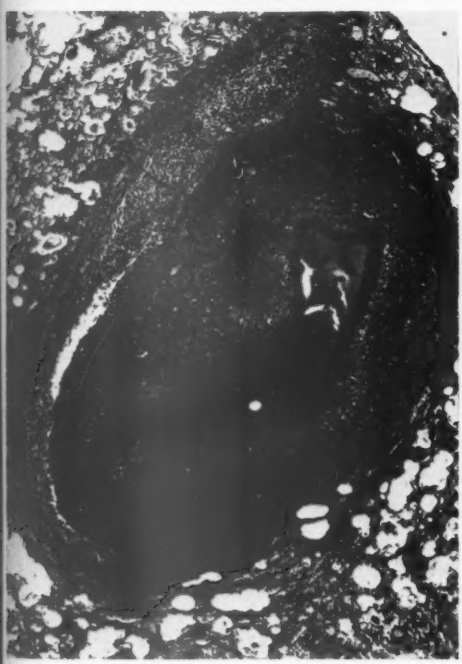

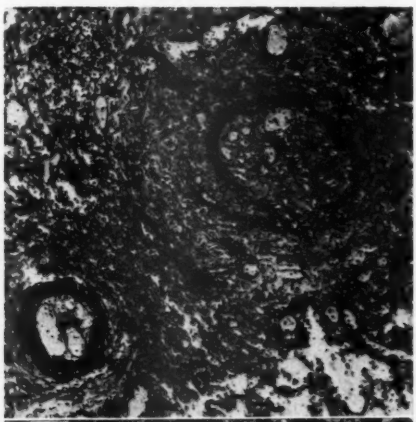

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HISTOGENESIS OF PAPILLARY CYSTADENOMA LYMPHOMATOSUM (WARTHIN'S TUMOR) OF THE PAROTID SALIVARY GLAND *

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For many years prior to 1910 authors sought to include the neoplasm known today as papillary cystadenoma lymphomatosum among the tumors of the lateral cervical cysts. In that year papillary cystadenoma lymphomatosum was recognized as a distinct entity for the first time (Albrecht and Arzt[1]), and final separation was attained from branchiogenic and dermoid cysts. Following this publication many names for the neoplasm were proposed, but with the well known paper by Warthin[2] in 1929, the designation of the tumor as papillary cystadenoma lymphomatosum was generally accepted.

The contribution of Albrecht and Arzt[1] should have crystallized the definition of this neoplasm, and to some extent clarified its histogenesis. Such has not been the case. It is believed that the greater part of the subsequent confusion has stemmed from: (1) the designation of any tumor in the cervical region with a cystic and papilliferous pattern as papillary cystadenoma, (2) the indiscriminate inclusion under the term adenolymphoma of the Warthin tumor along with other tumors (used in a broad sense) that occur in the parotid gland region[3-7] and consist of lymphoid and epithelial elements, and (3) the designation of the papillary cystadenoma lymphomatosum by many names derived from various concepts of its histogenesis (Table I). Because of this confusion, it has not been possible to collect all of the typical cases from the literature with accuracy.

In our survey, 163 acceptable cases have been found.[1-12,14-19,21-68] Some of these cases[8,18] have been included with hesitancy. This is true also of 2 of Lederman's cases (a 66-year-old male and a 42-year-old female).[56] There have been others for which the original articles have not been available; these have been included because those citing them gave evidence of being familiar with the neoplasm under consideration. Thus we have included cases by Ruiz,[69] cited by Niño[50]; Mosto[70] and Marvel,[71] cited by Duany[14] and Niño[50]; Bianchi and Pavlovsky[72] and Matsushima,[73] cited by Duany[14]; Putschar[74] and Wohlwill,[75] cited by Berner[55]; and Ehrlicher,[13] cited by Duany.[14] Some reported cases have been excluded,[76-79] primarily because the data recorded have not been considered adequate. Berner's[55] case 5 and Nicholson's[15] case 1 have been excluded for this reason. Some cases have been reported by differ-

* Received for publication, December 8, 1949.

ent authors; these include cases by Plaut [7] and Martin and Ehrlich,[26] and by Harris [25] and Swinton and Warren.[80] Whenever duplication has been recognized these cases have been included only under the original report.

Of the 180 acceptable cases (163 from the literature and 17 reported here for the first time) of papillary cystadenoma lymphomatosum, all

TABLE I

Names Applied to the Papillary Cystadenoma Lymphomatosum by Various Authors

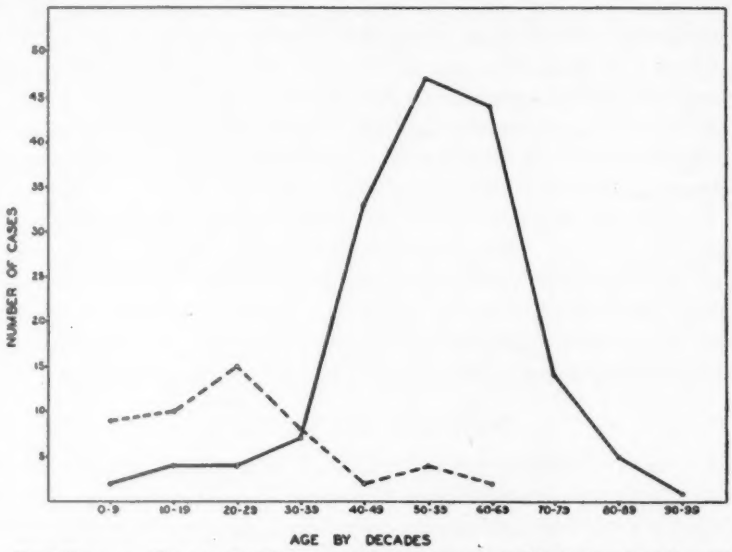
Authors	Diagnoses
Hildebrand[8]	Congenital epithelial cyst of the neck
Lecène[9]	Cystic adenoma of the parotid gland
Albrecht and Arzt[1]	Papillary cystadenoma in typical lymph node
Glass[10]	Branchiogenic papillary cystadenolymphoma
Ssobolew[11]	Branchioma
Feldmann[12]	Branchiogenic adenoma
Ehrlicher,[13] cited by Duany[14]	Papillary cystadenoma with lymphoid supporting tissue
Nicholson[15]	Cystic papillary adenoma
Mazza and Cassinelli[16]	Papillary cystadenolymphoma
Menetrier, Peyron, and Surmont[17]	Kyste amygdaloïde
Hickel[18]	Tumeur amygdaloïde polykystiques
Stöhr and Risak[19]	Cystadenolymphoma parotidis
Askanazy,[20] cited by Sternberg[21]	Adenoma branchiogenes cylindrocellulare cysticum
Warthin[2]	Papillary cystadenoma lymphomatosum
Bottin[22]	Papillary cystadenoma
Jaffé[23]	Onkocytoma
Kraissl and Stout[24]	Cystadenoma (orbital inclusion cyst)
Hall[3]	Adenolymphoma (orbital inclusion adenoma)
Harris[25]	Adenocystoma lymphomatosum
Martin and Ehrlich[26]	Warthin's tumor

were located in the region of the parotid gland except 5. One was from the submaxillary gland region,[32] and 4 were supposedly from the submaxillary salivary gland itself.[1,32,34,38] In 10 patients, the condition was bilateral. The sex was indicated for 141 males and 19 females, a ratio of roughly 7 to 1. The age distribution is shown in Text-Figure 1.

If the Warthin tumor is to be included in the general group of benign lympho-epithelial tumors designated as adenolymphoma, this should be done with discrimination. That all of the tumors included under this term are true neoplasms is far from certain. In the parotid salivary gland area, lymph nodes containing included salivary gland ducts (as will be described subsequently) may be the seat of chronic hyperplastic lymphadenitis secondary to some inflammatory process about the head or neck. Some of these may have been considered erroneously as true adenolymphomas. However, the tumor of Albrecht and Arzt,[1] which at the present time is designated as papillary cystadenoma lymphomatosum, is accepted as a true neoplasm. An additional factor that must be taken into consideration is the more aggressive character of some

true adenolymphomas (not Warthin tumors) which has led to the opinion that they may be carcinomatous.[62] A tumor of this character (3654–AX) has been seen in this laboratory.

No convincing examples of carcinomatous change in a Warthin tumor have been encountered in the literature or seen in our material. In Ssobolew's[11] case, carcinoma could be demonstrated in only one of



Text-Figure 1. The age distribution of Warthin tumors (solid line) and of branchial cysts and fistulas (broken line).

three pieces of tissue available for study. He could not establish a relationship between the carcinoma and the papilliferous cystadenoma lymphomatosum present in the remainder of his material. The author's photomicrograph of the carcinoma is not sufficiently clear to be evaluated. The clinical course could not be followed because of the early death of the patient from another cause. This case may be similar to case 7 of our series in which a Warthin tumor and a carcinoma were found in material removed from the left parotid region. However, no relationship could be demonstrated between the two. Hanford's[36] second case may be in this category also, as the evidence of carcinoma arising in the initial neoplasm cannot be considered proved. We cannot accept a diagnosis of carcinoma on the evidence submitted by Stöhr and Risak,[19] although this case has been so considered by some authors.[55,64] Gödel[46] and Lederman[56] have not established convincingly the presence of a Warthin tumor in their cases.

An evaluation of the characteristics of other adenolymphomas produces some interesting data, when contrasted with the features of the Warthin tumor. Twelve such cases have been gathered from the literature.[7,62,81,82] One has been considered as showing sarcomatous change in the supporting lymphoid stroma,[82] and one has been interpreted as carcinoma.[62] Seven of the 12 are reported as occurring in females, 4 in males, and the sex is not indicated in one. While no definite conclusions can be derived from the review of only 12 cases, these data are presented for what they may be worth as indicating dissimilarity when compared with true papillary cystadenoma lymphomatosum.

It is considered that the foregoing observations make it imperative to maintain the Warthin tumor as a distinct entity in the group of adenolymphomas that are true neoplasms.

It is our purpose to re-define the papilliferous cystadenoma lymphomatosum, and to evaluate the various concepts concerning its histogenesis. Particular attention has been given certain disputed histologic features of the neoplasm: the presence of ciliated epithelium, the presence of secretory capillaries, the presence of squamous epithelium, and whether the lymphoid elements represent true lymphnodal tissue.

Materials and Methods

The material utilized in this study has been arranged in four groups: human embryos, presumably normal parotid and submaxillary salivary glands, accepted branchiogenic residual structures, and neoplasms having the features of Warthin's tumor.

Through the courtesy of the Department of Anatomy of the University of Michigan Medical School, it was possible to examine serial sections of the head and neck of 18 embryos, varying in crown-rump length from 20 to 200 mm. This material constituted group 1. Attention was directed to the development of the parotid, submaxillary, and sublingual salivary glands.

The materials selected for the second group were divided into two parts. Part 1 consisted of 100 normal parotid salivary glands obtained at necropsy from patients dying of causes unrelated to the face, mouth, and neck. The ages at the time of death varied from the seventh month of gestation to 75 years. Thirty-five glands were from females, and 65 were from males. Approximately two-thirds of each gland was removed for study, and from each gland two blocks, on the average, were prepared. Eighty of the glands were fixed in formalin and 20 were fixed in absolute alcohol. The sections were stained with hemalum and eosin. This material has served as a basis for two previous studies.[83,84] Part 2

of this group was composed of 50 submaxillary salivary glands. Two of these were removed during post-mortem examination, and 2 presenting the features of chronic sialadenitis were removed surgically for that condition. The remaining 46 glands were included in the material from suprahyoid cervical lymph node dissection in patients with carcinoma of the face, lips, or mouth. All patients were adults and a majority were in the fifth and sixth decades. One or two blocks of tissue were available for study in each instance. All tissues were fixed in formalin and stained with hemalum and eosin.

Fifty branchiogenic cysts and fistulae were selected at random to constitute group 3 of this series. The variation in age of these patients was from 4 to 40 years (Text-Fig. 1). The cases were about equally distributed between the sexes. In the majority only one microscopic section was examined.

TABLE II

Twenty-three Examples of Papillary Cystadenoma Lymphomatosum Comprising Group 4 of the Material Studied

University of Michigan series				
Number	Identification of case	Age	Sex	Location
1	6630–AL	45	M	Right
2	5759–AN	68	M	Right
3	122–AR	65	M	Left
4*	1285–AS	63	M	Left
5	3989–AU	60	M	Left
6	5337–AV	70	M	Left
7	49–AW	61	F	Left
8	3807–AX	50	M	Right
9*	1542–AY	65	M	Left
10†	1431–LAD	45	M	
11†	4887–LAF	60	M	Left
12‡	3358–LAH			
13§	3306–LAK	50	M	Right
14	4230–LAQ	60		
15	7681–LAV	51	M	
16	9217–LAX	64	M	
17	1789–LAY	62	M	
18	4152–LAY	70	M	Right
19	6053–LAZ			
20	2450–LBA	45	M	
21	7653–LBA		M	
22‖	44–1193	50	M	Right
23‖	45–1964	55	M	

* Reported by Kerr.[28]
† Reported by Warthin.[2]
‡ Reported by Wendel.[34]
§ Reported by Odén.[41]
‖ From St. Joseph's Mercy Hospital.

Twenty-three examples of papilliferous cystadenoma lymphomatosum comprised the fourth group (Table II). Twenty-one cases were from the Department of Pathology of the University of Michigan. Nine of these were from the University Hospital and 12 cases from the extra-

mural diagnostic service. Two cases were from the laboratory of pathology, St. Joseph's Mercy Hospital. Six of the 23 have been reported previously. Cases 10 and 11 were the basis of Warthin's report [2] in 1929. Case 12 was described by Wendel [34] in 1930. This was the only instance in this series of papillary cystadenoma lymphomatosum supposedly arising in the submaxillary salivary gland. Case 13 was reported by Odén [41] in 1935. Cases 4 and 9 were mentioned by Kerr [63] in 1947. The 11 cases from the University and St. Joseph's Mercy hospitals were from the parotid gland or its immediate vicinity. The cases from the outside diagnostic service were, so far as known, all from the same area. The original tissues in group 4 were fixed in formalin and stained with hemalum and eosin. From the material from the University Hospital, sections 5 μ in thickness were counter-fixed in slightly acidified Zenker's solution at room temperature for 6 to 8 hours, then stained by the Goldner [85] modification of the Masson trichrome technic. Mallory's aniline blue, orange G, and acid fuchsin technic was found to be unsuitable because nuclear detail was masked.

Embryology

The embryology of the major salivary glands must be considered briefly, with only those features that contribute to the elucidation of the histogenesis of the Warthin tumor given detailed presentation. The reader may refer to standard texts on developmental anatomy [86-90] for additional information.

The anlagen of the parotid and submaxillary salivary glands appear in man during the sixth week of embryonic life.[87-90] The buccal sulcus gives rise to two derivatives: the orbital inclusion from a more posterior position [87,88,91] (Fig. 1), and the parotid anlage from a more anterior portion [87,88] (Fig. 2). The existence of the orbital inclusion in man has been denied.[62] The submaxillary salivary gland anlage, and, later, those of the sublingual glands, arise from the alveololingual sulcus.

Schulte [87] and Carmalt [88] described the orbital inclusion, once it has been separated from the oral epithelium, as being displaced laterally and coming to lie close to the muscles of mastication. It progresses caudally, medial to the masseter muscle (Fig. 3), to a position against the internal pterygoid muscle. The orbital inclusion is derived from a segment of the buccal sulcus, which subsequently gives rise to the orbital glands in some carnivora. The significance of the presence or absence of an orbito-parotid interval or an orbito-parotid area of the buccal sulcus between the parotid gland and orbital inclusion anlagen need not

be considered at this time. Occasionally separate glandular masses are found in relation to the intramural and buccinator portions of the parotid duct in man.[87,88] These do not communicate with the parotid duct, but open by small, independent, separate orifices on the oral mucosa posterior to the opening of the parotid duct. When well developed, a series of these glands may be imbedded in the fat pad medial to the masseter muscle. This series represents the reduced human representatives of the orbital glands in some carnivora.

The epithelial anlage of the parotid gland may be traced in a caudal direction from its point of origin. The epithelial cylinder traverses the external surface of the masseter muscle (Fig. 3), to the retromasseteric locus of the parotid gland. The duct ramifies freely into cell cords, which eventually differentiate into branching ducts and alveoli. Aggregates of "round cells" are noted in the parotid gland region in embryos of a crown-rump length of 20.5 mm. At this stage it is not possible to identify these elements as lymphocytes. Lymphoid tissue was identified in this area in an embryo with crown-breech length of 60 mm. and in other fetuses through the oldest specimen in this series which had a crown-rump length of 200 mm. In this material lymphoid tissue was observed to exist as lymphoid aggregates and lymph nodes in embryos of 89, 105, and 200 mm. crown-breech length (Figs. 4 and 5). It was particularly impressive to note the absence of lymphoid elements in the submaxillary and sublingual glands of the same embryos.

An additional significant embryologic observation has to do with the comparative dispatch with which the organoid integrity of the salivary glands is established. In embryos as young as 7½ weeks the submaxillary gland gave the impression of developing as a unit. From this stage through that of a 14-week embryo there was a tendency for the submaxillary salivary elements to appear as a compact organoid entity, developing as a unit within a capsule formed by the condensation of mesenchyme (Fig. 6). On the other hand, the parenchymal elements of the parotid gland were arranged in a loose manner, and had a "sprawled out" appearance (Fig. 7). No evidence of mesenchymal condensation to form a capsule was apparent in our specimens until the 105 mm. (14 weeks) stage. In some of the slightly older embryos (100, 105, 200 mm.) there were lymph nodes containing salivary gland ducts (Fig. 5) inside and outside of the capsular area of the parotid gland. The sublingual gland, as far as capsular development is concerned, also appeared to evolve in a more closely integrated manner than the parotid gland.

The Control Series of Parotid and Submaxillary Salivary Glands

Parotid Gland

In general, the results of our study of normal parotid salivary glands are in agreement with the descriptions in the standard textbooks of histology. One of the more significant findings in 25 of this group of 100 was the presence of lymphoid aggregates surrounded by fibrous connective tissue stroma within the gland proper. In approximately two-thirds of the 25 cases, some or all of the characteristic features of lymph nodes (differentiation into cortex and medulla, lymphatic sinuses beneath the capsule and in the medullary area) were apparent. The aggregates varied in volume within the usual range of lymph nodes. Whether some of these lymphoid aggregates should be designated as lymph nodes has been debated.[26,46] It is not pertinent to enter into the discussion here. Suffice it to say, that lymph nodes in other areas of the body may exhibit histologic features which are not in complete accord with the usual morphologic pattern. In unquestioned lymph nodes of the parotid gland, the lymphnodal features in many instances are not as distinct or as clearly defined as in lymph nodes from some other regions. This may be due in part to the increased development of fibrous connective tissue stroma in the lymph nodes of the parotid gland area. Henceforth, the larger encapsulated lymphoid aggregates will be referred to as lymph nodes.

In 6 of the 25 cases, parotid gland ducts were identified in lymph nodes (Figs. 8, 9, and 10). The ages of the patients were the 7th month of gestation and 22, 34, 37, 54, and 60 years. In some instances a lobule of the gland could be seen extending into the hilus of a lymph node (Fig. 11). This feature was noted also in a few of the remaining 19 cases. The fact that all of these 6 cases were in males was considered significant, since the ratio of males to females with lymph nodes in the parotid gland was reasonably close statistically to the sex ratio for the total 100 cases.

The epithelium lining the duct system of the parotid gland was found to correspond to the descriptions in the literature with few exceptions.[92] Ciliated epithelium could not be demonstrated in any of the ducts. In the glands of patients of more than 50 years of age, oxyphilic granular cells[88] (pyknocytes[93] or oncocytes[94]) were present as noted previously by Meza-Chávez.[88] Ducts showing transition of the epithelium from the usual type to the oxyphilic granular type were demonstrable (Figs. 12 and 13). In a few cases, ductal epithelial cells were seen that corresponded to a type previously described[92] as occurring in tumors of the

salivary glands (Fig. 14). These cells, designated "clear cells" in the past, should be considered in the majority of instances as ductal epithelial cells partially or wholly devoid of cytoplasmic granules. In one example (case 9), such cells formed the sole type of epithelium lining several excretory ducts. In this case, transitions from ductal epithelium of the oxyphilic granular type to the clear type could be demonstrated. Such a change was apparent also in the acinar cells. Since the integrity of the nuclei was maintained as a rule, these cytoplasmic changes were interpreted as being related to some unusual metabolic or secretory activity, rather than to retrogressive changes. Occasionally, as among the oxyphilic granular cells, clear cells could be seen with indented or pyknotic nuclei. With the exception of the character of the cytoplasm, these cells corresponded in physical appearance to the oxyphilic granular cells. This was interpreted as indicating that the clear cells of the ductal epithelium probably are derived from the oxyphilic granular cell variant of the usual ductal epithelium, and that the oxyphilic granular cell and the clear cell are both normal variants of the ductal epithelium, the oxyphilic granular cell being related to the ageing process, and the clear cell being a manifestation of hypersecretion or some other metabolic change.

At infrequent intervals ducts were seen lined by true goblet cells. These were distinctly different from the cells described in the preceding paragraph, and they appeared to be more closely related to the usual parotid ductal epithelium of younger individuals. On occasions these elements have been described in the Warthin tumor.[46,55] They may be related more closely to the adenocystoma mucipare and muco-epidermoid tumors.[55,92,95,96]

Submaxillary Salivary Gland

The submaxillary glands included in this group could not be considered, in the strict sense of the word, normal. As previously noted, some of these glands were in the zone of lymph drainage from carcinomas of the face, mouth, or lips; others were examples of chronic sialadenitis. Accordingly, these glands could be expected to have a greater degree of lymphocytic infiltration than would be found in strictly normal glands. The degree of lymphocytic infiltration was graded from 0 to 3. Cases graded 0 exhibited very few lymphocytes, and those graded 3 showed germinal centers. Seventy per cent of the 50 submaxillary salivary glands examined were classified under 0, 18 per cent as 1, and 8 and 2 per cent as 2 and 3, respectively. The more extreme examples were cases of chronic sialadenitis. Unlike the findings in the parotid gland, no lymph nodes or encapsulated lymphoid aggregates were found.

In nearly all instances, the lymphocytic infiltrations, when present, were periductal and of an ill defined nature.

This group of glands included one with dilated ducts lined by tall, non-ciliated, eosinophilic, columnar epithelium. Low papillations projected into the lumina (Fig. 15).

In contrasting the groups of parotid and submaxillary salivary glands examined, two distinct differences were apparent. In the parotid gland, lymph nodes and encapsulated lymphoid aggregates were relatively common, whereas no such structures were observed in the submaxillary glands. Small, ill defined, periductal aggregates of lymphocytes were noted frequently in the parotid gland, but were relatively uncommon in the submaxillary gland except in instances of obvious chronic inflammation. The basic difference in the character of the two glands corresponded closely with the findings in the embryos. Oxyphilic granular cell variation in the ductal epithelium was not as pronounced as in the parotid glands.

Branchiogenic Cysts and Fistulas

The branchiogenic derivatives examined were all from the area anterior to the sternocleidomastoid muscle, and near or below the ramus of the mandible. The primary interest in these structures was in the character of the epithelium. In all but 9 of the 50 examples, the epithelium was wholly of stratified squamous type. In 9 specimens, the cysts or fistulas were lined in part by stratified squamous and in part by pseudostratified, ciliated, columnar epithelium, or by pseudostratified, ciliated, columnar epithelium alone. There were no similarities, either in staining qualities or in nuclear arrangement, between the columnar epithelium of the branchial cysts and fistulas, and that of the Warthin tumor.

Berner[55] criticized the branchiogenic concept of the origin of the papilliferous cystadenoma lymphomatosum on the basis that the parotid anlage in no way comes in contact with the pharyngeal pouches. One case in our material presented a branchial fistula lined by respiratory and stratified squamous epithelium, which extended through the lower pole of the parotid gland. The histologic features of this structure are distinctly different from those of the Warthin tumor.

Morphology

It would be repetitious to describe in detail each of the 23 cases of papilliferous cystadenoma lymphomatosum in this series. In the presentation that follows, it is our purpose to review some of the characteristics of the neoplasm, and to take exception to some of the previously

recorded statements. It is intended to emphasize those histologic features that are of importance from the point of view of histogenesis.

The gross findings in our series were limited to the 9 University Hospital cases. The factual data agreed with the characteristics of the neoplasm as recorded in the literature. As others have noted, there was no constant relationship between the duration of symptoms and the size of the tumor. None of the neoplasms reached the size of the tumor described by Callender[97] in 1929. All of the tumors were unilateral.

A thin fibrous capsule surrounding most of these neoplasms could be identified in the microscopic sections. In the one exception (case 6), the neoplastic tissue appeared to extend into the normal parotid gland. These tumors consisted, as a rule, of an admixture of papilliferous epithelial elements lining cysts and of lymphoid stroma. In the solid epithelial areas, the epithelium was in a medullary and/or tubular pattern. The epithelial cells were usually of a tall, columnar, eosinophilic variety, with a basal layer of polyhedral cells (the type seen in many of the adenomas). These have been designated as oxyphilic granular cells,[83] oncocytes,[35] or pyknocytes.[93] These cells were not an invariable finding, as all variations could be demonstrated including cells that compared favorably with the usual parotid ductal epithelium. The average cell tended to have opaque homogeneous-appearing cytoplasm in hemalum and eosin preparations. In many instances fine and coarse pink granules were apparent. The granules stained either greenish gray or red in Masson trichrome preparations. Case 3 presented an extremely granular epithelium. In hemalum and eosin preparations, the staining characteristics varied from pale pink in "healthy" cells, to an orange-red in cells undergoing retrogressive change.

In approximately one-third of the cases there could be seen occasional, and in 2 instances numerous, epithelial cells almost entirely devoid of cytoplasmic granules (Fig. 16). The cell-outlines and nuclear details were well preserved. This feature was exemplified to an extreme degree in case 9. These cells contained no stainable lipid in the sudan III stain. The nuclei of the epithelial cells were oval as a rule, and located in the luminal one-third of the cell body. In the columnar cells the long axis of the nucleus usually was vertical, and in the polyhedral cells the long axis, in the majority of instances, was horizontal with reference to the basement membrane. In the latter cells, the nucleus occasionally was spherical. There was usually a visible nucleolus, and the chromatin was finely clumped.

The secretory activity of the epithelial cells aroused much interest, primarily because of the reported presence of intercellular secretory

capillaries.[23,45] The secreted material was chiefly of a pink granular quality in the hemalum and eosin preparations. Occasionally, rounded eosinophilic masses of secretion were present. In well preserved areas, the secreted material appeared to arise as rounded masses from the free surface of the superficial epithelial layer (Fig. 17). In the Masson trichrome preparations, such masses stained varying shades of green to brown. Later in their evolution, the secreted masses broke away from the surface of the cell and presented the characteristic granular appearance. Scattered at irregular intervals, and occasionally grouped in twos, threes, or fours, epithelial cells were seen in which the nuclei were pyknotic and deeply staining (Fig. 18). The cytoplasm of these cells stained a deep pink in contrast to the surrounding epithelium. With the Masson trichrome technic, such cells stained deep orange or red, and were delineated sharply from the neighboring epithelial elements. These cells were narrow as if horizontally collapsed, and in instances the attenuated basilar portion extended to the basement membrane. On occasion they were seen in the process of being extruded into the lumina of the cystic spaces (Fig. 19), after which they constituted a part of the secretion-complex. This feature has been described as epithelial secretion occupying secretory capillaries.[23,45] The presence of pyknotic nuclei negates this interpretation.

In the series of Warthin tumors available for study, the characteristics of the epithelium ran the gamut of the variations apparent in the ducts of normal parotid glands. Epithelium was seen that compared favorably with the usual ductal epithelium (Fig. 20), through the types characterized by Hamperl[35] as "Übergangsformen," to well developed oxyphilic granular cells or oncocytes (Fig. 21). The "clear cell" variation of the oxyphilic granular cell (Figs. 16 and 18) also was found in some cases (7 and 9). As a general rule, the epithelium was arranged in two layers, a superficial columnar type and a second row of polyhedral cells. In some areas the epithelium consisted of more than two layers. Metaplasia of the usual columnar epithelium to stratified squamous occurred, but was rare. Ciliated epithelium was not observed.

The supporting lymphoid tissue showed the essential characteristics of a lymph node (cortex, lymph sinuses, and capsule) in some cases (Fig. 22). Sections of a neoplasm selected to demonstrate these findings, should be those taken through the capsule with a sufficiently adequate zone of uninvolved lymphoid tissue to make demonstration of these characteristics possible. When the epithelial portion of the tumor extended to the capsule with only small islands of lymphoid tissue apparent, these characteristics were obliterated. Consequently all of the

tumors were not satisfactory for the demonstration of these manifestations. The medulla usually was not apparent, as it was occupied for the most part by the epithelial portion of the neoplasm. In evaluating the lymphnodal component of the tumor, it was found desirable to utilize lymph nodes from the parotid gland as controls.

In the majority of cases, areas of inflammation were present, which were apparently secondary to the retained epithelial products in the cystic spaces. These areas were characterized by the presence of polymorphonuclear eosinophilic and neutrophilic leukocytes, plasma cells, histiocytes, and scar tissue (Fig. 21). The lymphoid tissue in the neoplasm tended to show the same propensity for fibrous connective tissue formation that had been noted in the lymph nodes of the parotid glands of older individuals. The scar tissue in these tumors tended to occupy a position parallel, and immediately adjacent, to the epithelium lining the cysts. In case 8 (Fig. 23) a polypoid mass of young scar tissue was protruding into one of the cysts. In addition there were hyalinized areas of old scar tissue. Manipulation of these masses by the patient may have contributed to some of these findings.

In summary, the histologic features agree with those previously described with certain exceptions. Neither cilia nor secretory capillaries can be demonstrated in our cases. The character of the epithelial cells shows a gradient from those closely similar to the usual parotid ductal epithelium, to the large granular and clear types of oxyphilic granular cells. There is also a variation in the degree of epithelial proliferation. The majority of cases present cysts lined by epithelium two layers in thickness; others exhibit exuberant cellular proliferation with secondary papillae. It is our conclusion that the epithelial cells secrete granular material into the lumina of the cysts and, on reaching what might be termed maturity, are extruded into the cystic spaces. The lymphoid portion of the neoplasm is derived from a lymph node or an encapsulated lymphoid aggregate, which is supplemented later by an inflammatory component.

Histogenesis

The embryologic development and certain of the histologic characteristics of the submaxillary and parotid salivary glands have been considered. There is no feature of the papillary cystadenoma lymphomatosum for which the homologue has not been demonstrated in the parotid gland. All gradations have been observed, from ducts with lumina altered but little, if any, and lined by the usual epithelial cells, oxyphilic granular cells, or combinations of these types, to varying degrees of ductal dilatation with papillae of oxyphilic granular cell epithelium.

These variations have been more striking in the intralobular ducts of the parotid gland but have not been unusual in the interlobular ducts. On occasion oxyphilic granular cells are found in nests. The cellular pattern varies from simple medullary clusters to tubular formations. Foci are seen in which the pattern is mixed (Fig. 24). These clusters are situated usually in gland lobules, and vary in size from small nests to masses satisfying the requirements for a neoplasm. The latter extreme has been termed an oxyphilic granular cell adenoma or a type of oncocytoma.[83] The pure epithelial areas of many of the Warthin tumors (Fig. 25) consist of solid masses of somewhat polyhedral eosinophilic cells, that exist in medullary nests and demonstrate a tendency to form tubules. These areas present a histologic picture almost identical with some of the oxyphilic granular cell adenomas. The epithelial component of the papilliferous cystadenoma lymphomatosum differs, in general, from the pure adenoma only in the pattern of growth. These observations are believed to show the relationship between the two neoplasms, and to denote that the ductal epithelium, primarily at the intralobular level, contributes to the origin of both neoplasms.

That the epithelium in the majority of Warthin tumors is of the oxyphilic granular cell type is considered to be fortuitous. This alteration in the epithelium is not a neoplastic prerequisite, as all possible epithelial variations have been observed in the tumors examined. The age range in which the neoplasm occurs may contribute to the preponderance of oxyphils.

In approaching the fully differentiated papilliferous cystadenoma lymphomatosum, it may be well to consider a tumor believed to occupy a position between that neoplasm and the simple inclusion of ducts in lymph nodes. This may be considered a variant of the Warthin tumor, as it somewhat resembles case III of Gaston and Tedeschi.[62] Our case 12307-LAZ is an example of a polycystic parotid tumor with lymphoid stroma (Fig. 26), in which the multi-layered lining epithelium consists of a surface layer of cuboidal cells with the same staining characteristics as the usual ductal epithelium. In areas the epithelium is flattened to such an extent that it is not unlike stratified squamous epithelium. There is an ill defined papilliferous pattern. Such a tumor may be representative of some others included with the Warthin tumors in the literature.[8,18]

As noted in the control material, the mixture of epithelial and lymphoid elements characteristic of the papilliferous cystadenoma lymphomatosum was observed only in the parotid gland, and its immediate vicinity. Such structures were not observed in relation to any of the

other major salivary glands. If salivary gland ducts occur at all in lymph nodes in relation to the submaxillary and sublingual salivary glands, it must be considered a rare association. All of the Warthin tumors included in this report and in respect to which location was given, were located in the parotid gland or its immediate environs with 5 exceptions. It was implied that 4 of these were removed from the submaxillary salivary gland (Spitznagel,[5,32] Gödel,[5,32] Wendel,[34] and Steinhardt[38]). Albrecht and Arzt[1] reported one from the submaxillary region, but the relationship was not considered sufficiently definitive to permit tabulation as a tumor arising in the submaxillary gland. Martin and Ehrlich[26] did not accept the tumors of Albrecht and Arzt, Spitznagel, and Wendel as of submaxillary origin. "None of these authorities actually stated that the tumors were found in the substance of the submaxillary gland or attached to its anterior surface. The fact that they have not done so indicates that they have not appreciated the fact that the anteroinferior aspect of the tail of the parotid lies in contact with the posterior superior aspect of the submaxillary salivary gland, although the deep cervical fascia separates the two." It appears highly probable that all of these neoplasms arise in the parotid gland or its immediate vicinity. If any occur in the submaxillary gland, they may be expected to be histologically similar to the neoplasm reported by Steinhardt.[38] This tumor has been described as consisting of delicate papillae lined by cubical epithelium, chiefly in a single layer. His photomicrograph shows a relatively smaller quantity of lymphoid tissue than is seen in the usual case from the parotid region.

It seems apparent, therefore, that the Warthin tumor is the result of the neoplastic proliferation of ducts. These epithelial components are clothed in lymphoid tissue. The lymphoid tissue, in instances, exhibits the characteristics of a lymph node. Considering the histologic sections of the individual tumors alone, there is no clear evidence to disprove absolutely that in individual instances Warthin tumors may not arise in ducts outside of lymphoid tissue, with a secondary infiltration of lymphocytes on an inflammatory basis. The genesis of this inflammatory response has been described previously, and the possibility is supported by the fact that, on occasion, phagocytes in the surrounding stroma may contain material similar to that in the cystic spaces.

The normal function of the lymph node as a filter is impaired due to the interference with filtration by the included epithelial structures. The integral lymphnodal characteristics are distorted under these circumstances; however, they can be demonstrated in some instances if the tumor is fortuitously sectioned in such a way that these structural

manifestations are not concealed by the epithelial elements and the inflammatory response.

Some of the papilliferous lymphomatous cystadenomas occur within the parotid gland, and others outside of the gland proper in the vicinity of its capsule. Observations of the gland during its embryonal development explain the occurrence of the neoplasm in paraglandular locations. It has been noted that the parotid gland, unlike the submaxillary salivary gland, does not develop as a closely aggregated integral unit. When the capsule of the gland finally condenses at a later stage, lymph nodes at the periphery, into which ducts have penetrated, may be isolated outside the glandular area. In such cases the ducts penetrating the lymph nodes must be interrupted, with the lymph nodes retaining the distal portions. A neoplasm arising in such a lymph node would be located outside of the parotid gland proper.

Discussion

Several concepts of the histogenesis of lateral congenital cysts of the neck have been proposed.

Wenglowski,[98] in 1912, following extensive embryologic and anatomical research, related the origin of cervical cysts and fistulae to a persistence of the thymic anlage and the thymopharyngeal canal. Delanglade *et al.*,[77] in 1914, described additional cases. These authors indicated the presence of elements having great similarity to Hassall's corpuscles. The spaces in these structures were described as lined by stratified squamous and ciliated columnar epithelium. The origin of such epithelial structures from remains of the embryonic pharyngeal pouches and gill furrows was proposed by some (Ssobolew,[11] Feldmann,[12] Askanazy,[20] Spitznagel and Gödel[32]), and from heterotopia of pharyngeal endoderm by others.[2]

Gaston and Tedeschi[62] considered that the lack of ciliated columnar epithelium in the Warthin tumor is not of importance in eliminating the sources of origin mentioned in the preceding paragraph. It seems apparent from our studies that it *is* an important factor. The cysts and fistulae from branchial remains are lined invariably by either stratified squamous or ciliated columnar epithelium. Among the Warthin tumors examined, there has not been a single convincing instance of cystic spaces lined by ciliated columnar epithelium, and squamous metaplasia is exceedingly rare. The same reasoning may be applied to the concept of arrested thymic anlage or the so-called thymopharyngeal canal. In addition, no structures resembling Hassall's corpuscles have been observed in the lymphoid tissue. The age incidence of patients consulting

physicians for branchial cysts and fistulae is decidedly different from that of those with Warthin tumors.

We believe that the theory advanced by Kraissl and Stout[24] which related the orbital inclusion or the "organ of Chievitz" to the Warthin tumor can be eliminated. If this structure contributed to the genesis of the Warthin tumor, such neoplasms should appear more frequently in the area medial to the masseter muscle and mandible, and more anterior than the parotid area. As far as can be determined, no papilliferous cystadenomata lymphomatosa have been reported in the area described.

The original concept of Robert Meyer[99] that epithelium-like linings of this type represent hypertrophic endothelium in diseased lymph nodes may be discounted. The epithelial nature of this portion of the tumor has been accepted generally.

The oncocytic concept of origin[28] does not adequately explain all of the features of the neoplasm, in that it does not account for the Warthin tumors in which the epithelial component is not of the oncocytic type. In addition, these cells have been described in other locations,[35,46] but Warthin tumors have been described only in the parotid area.

These neoplasms present a good prognosis, and, with rare exceptions, no other neoplasm has been described in the head and neck region of patients who bear them. Such considerations eliminate the possibility that these neoplasms are metastatic carcinomas.

It is our opinion that a careful microscopic evaluation of any Warthin tumor makes it possible to place the neoplasm in one of two histogenetic groups.

1. *Neoplasms arising from parotid ductal epithelial elements included in lymph nodes,* such inclusion having been described previously by Neisse,[100] Löwenstein,[101] Lubarsch,[102] and Bairati,[103] and verified in our material. Neisse and Bairati have described similar structures in the submaxillary region. This is essentially the original concept of Albrecht and Arzt.[1] As these writers have indicated, histologic examination of the neoplasm reveals the close relationship between the parotid gland and the tumor. Small salivary gland ducts are found in the capsule of the neoplasm and in the neighboring stroma. There is a striking similarity of the epithelial elements of the neoplasm, and the epithelial lining of the ducts of the parotid gland. "Es liegt daher nahe, auf Grund dieser so innigen Beziehungen wenigstens für unsere Geschwülste eine histogenetische Beziehung zu den Speicheldrüsen als möglich anzunehmen, entweder so, dass bei der ersten Differenzierung der Speicheldrüsen aus dem Entoderm der Mundbucht Gewebskeime der Speicheldrüsen aberriert und dann in Lymphknoten eingeschlossen worden

wären, ... Somit stellen unsere beiden Fälle gewiss zwei Beispiele von Gewebsverirrung oder Dystopie, wie dies R. Meyer neuerdings bezeichnet . . . ."

2. *Tumors due to the neoplastic proliferation of parotid ductal epithelium and the concomitant accumulation of lymphoid tissue in a manner previously described.* As indicated by some,[26,62] one of the primary objections to the first concept is that essential lymphnodal characteristics cannot be demonstrated, and for this reason it has been denied that the lymphoid elements represent lymph node. It is our contention that these characteristics can be demonstrated. It is agreed that there is a chronic inflammatory element in the stroma that is essentially of a lymphocytic nature. The propensity of the major salivary glands to develop lymphocytic accumulations in the stroma has been indicated by others.[28] Some have stated that the presence of the oxyphilic granular cell variation *per se* stimulates the accumulation of lymphoid elements.[35] It is our opinion that the collections of lymphocytes ordinarily seen around ducts as part of the process of inflammation contribute to the bulk of the neoplasm and aid in the distortion of the normal lymphnodal architecture. Study of some of these neoplasms in their early formative stages justifies the inclusion of this second group. The lymphoid tissue of these small neoplastic proliferations resembles that in some of the fully developed tumors, in that lymphnodal characteristics cannot be demonstrated. The epithelial elements, in these instances, develop in an aggregate of lymphoid tissue. As the tumor expands and the parenchyma of the glandular lobule undergoes atrophy, the trabeculae condense as a capsular structure around the periphery.

Summary

The development of the major salivary glands was studied in 18 embryos varying in crown-rump length from 20 to 200 mm. (7 to 21 weeks). Structural features were re-examined in 100 normal parotid glands from patients ranging in age from 7 months of gestation to 75 years; and in 50 submaxillary glands derived chiefly from patients in the fifth and sixth decades. Fifty branchiogenic cysts and sinuses from patients ranging from 4 to 40 years were examined also. Finally 23 examples of papilliferous cystadenoma lymphomatosum were studied.

The following conclusions have been derived from this study: The papilliferous cystadenoma lymphomatosum and the oxyphilic granular cell adenoma are related neoplasms that differ only in pattern and supporting stromal elements. Both neoplasms are derivatives of the epithelium of the excretory duct radicles of one of the major salivary glands. The Warthin tumor is considered to be a neoplasm of the pa-

rotid salivary gland and its immediate vicinity. The origin of this tumor is (1) in the neoplastic proliferation of parotid ducts included in lymph nodes. This opinion is based primarily on the finding that lymph nodes containing ducts have been observed, without doubt, only in the parotid gland area. The essential lymphnodal characteristics of the lymphocytic collections can be demonstrated in selected cases of neoplasm. (2) After origin of the neoplasm in the ductal epithelium of the parotid gland, the lymphoid tissue may be that of an inflammatory lymphoid aggregate.

The intimate relationship between the inferior portion of the parotid gland and the posterior portion of the submaxillary gland may be responsible for erroneously attributing neoplasms occurring in this area to the submaxillary gland.

That oxyphilic granular cells or oncocytes constitute the epithelial component of the Warthin tumor in the majority of cases is not considered to be of primary significance. It is thought that this epithelial variant does not constitute a neoplastic prerequisite, although this type of epithelium may be more disposed to neoplastic change than is the usual ductal epithelium.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 110

FIG. 1. A frontal section through the head of a human embryo (crown-rump length, 58 mm.). The orbital inclusion may be seen in the upper right corner as a small, dark knob of cells arising from the buccal sulcus. The mass in the lower left corner is the lateral superior portion of the tongue. Hemalum and eosin stain. × 100.

FIG. 2. A frontal section through a more anterior area of the embryo illustrated in Figure 1. The origin of the parotid gland is represented by the bud of dark staining cells arising from the buccal sulcus in the upper right corner of the illustration. The lateral superior surface of the tongue may be seen in the lower left corner. Hemalum and eosin stain. × 100.

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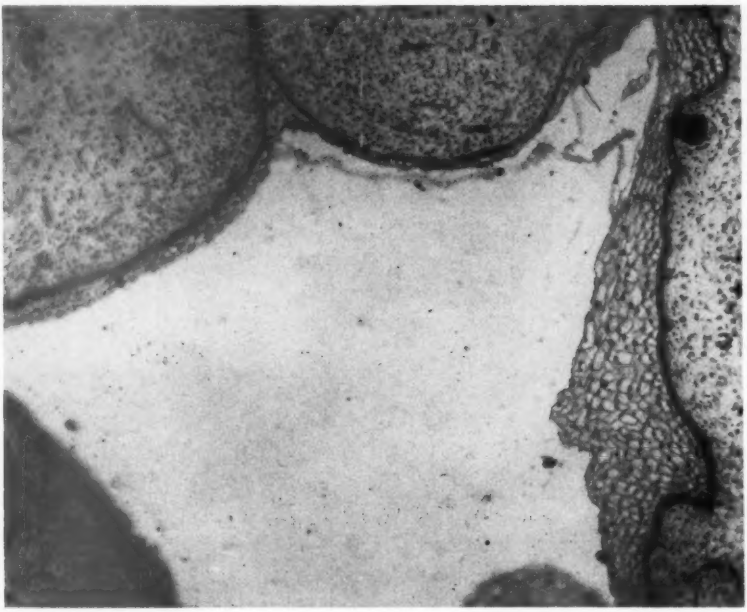

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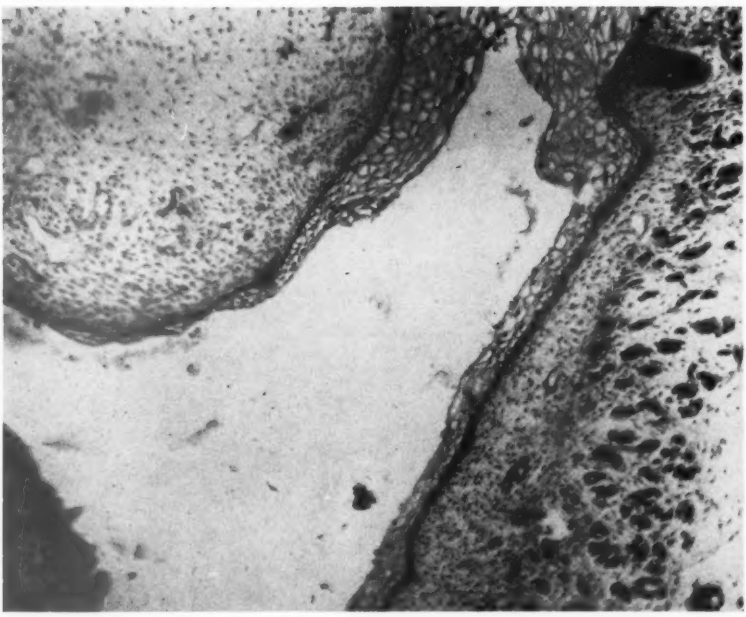
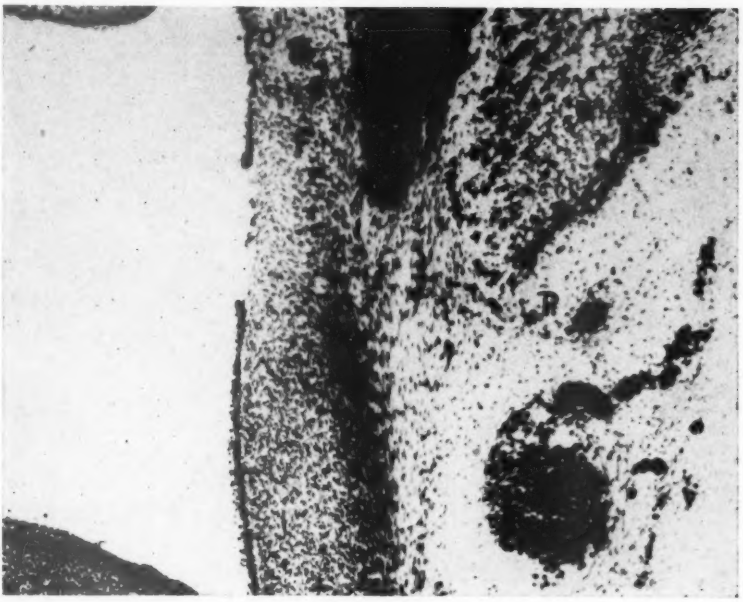


Plate III

Fig. 3. A frontal section of an embryo (c.-r.l., 28 mm.) through an area more posterior than that of either Figure 1 or Figure 2. O indicates the orbital inclusion lying medial to the muscles of mastication, and the small, dark mass near P is the parotid which has assumed a more lateral position. The lateral superior margin of the tongue may be seen in the lower left corner. Hemalum and eosin stain. × 100.

Fig. 4. Parotid ducts included in a lymph node from the parotid region of an embryo of 200 mm. crown-rump length. Hemalum and eosin stain. × 100.

3



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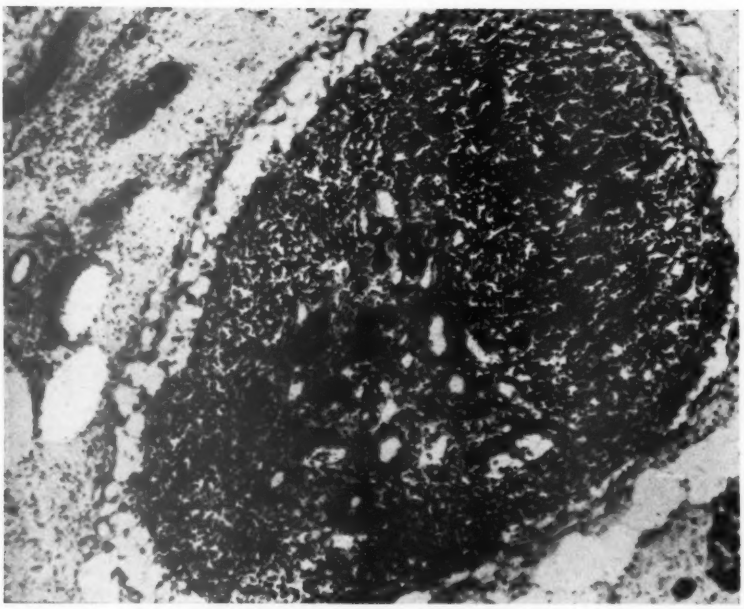


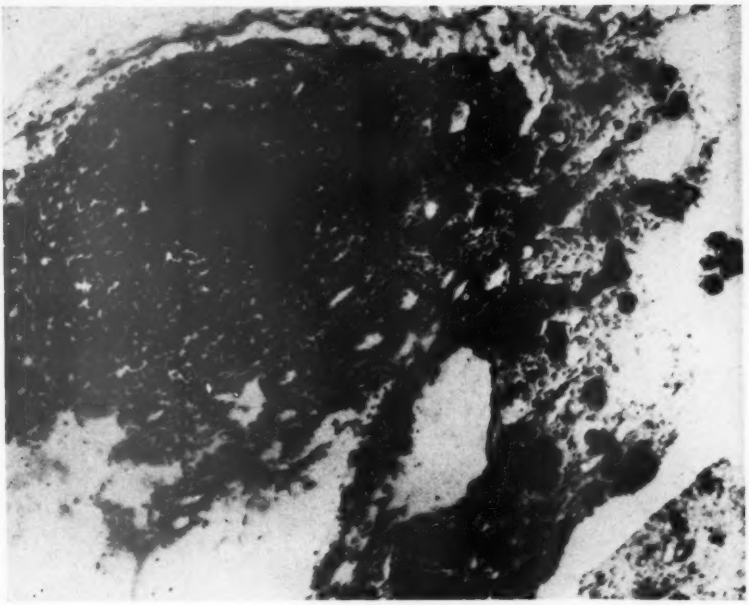
Bryant and Thompson Histogenesis of Papillary Cystadenoma Lymphomatosum

PLATE 112

FIG. 5. Parotid ducts included in a lymph node from the parotid region of an embryo of 105 mm. crown-rump length. Hemalum and eosin stain. × 100.

FIG. 6. The submaxillary gland of an embryo (c.-r.l., 58 mm.), demonstrating the tendency of this gland to be encapsulated and to develop as an organized unit. Hemalum and eosin stain. × 100.

5



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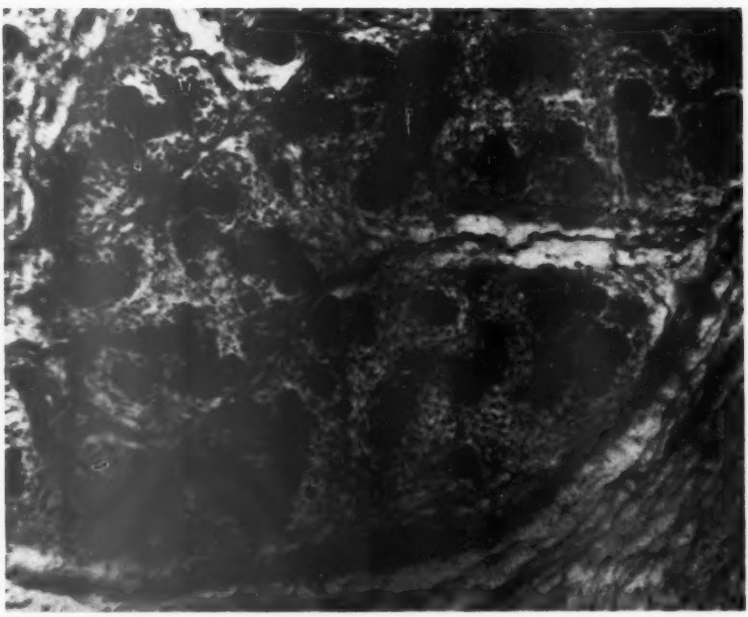

Bryant and Thompson Histogenesis of Papillary Cystadenoma Lymphomatosum

PLATE 113

FIG. 7. The parotid gland from the same embryo illustrated in Figure 6. The absence of a capsule may be noted, as well as the "sprawled out" appearance of the developing parenchyma. Hemalum and eosin stain. × 100.

FIG. 8. Parotid ducts in a lymph node from the parotid gland region of a fetus in the seventh month of gestation. Hemalum and eosin stain. × 200.

FIG. 9. Parotid ducts in a lymph node from the parotid gland region of an adult. Hemalum and eosin stain. × 240.

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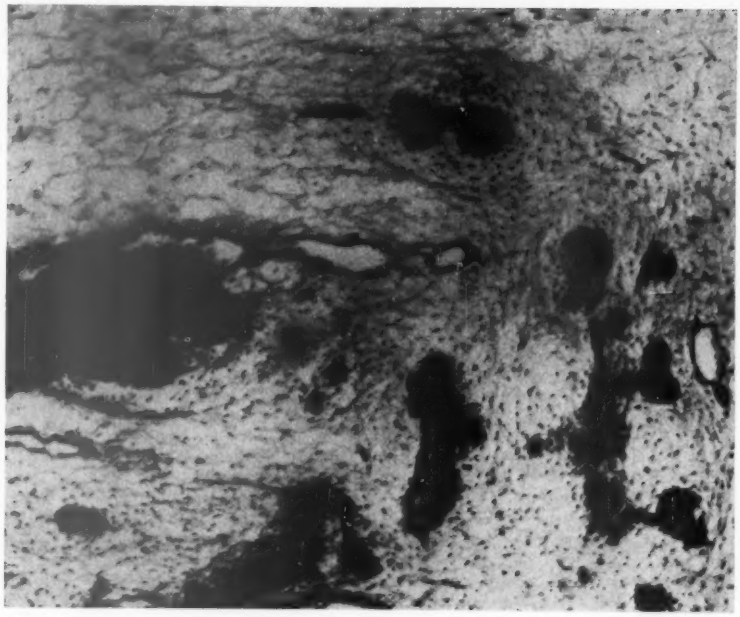

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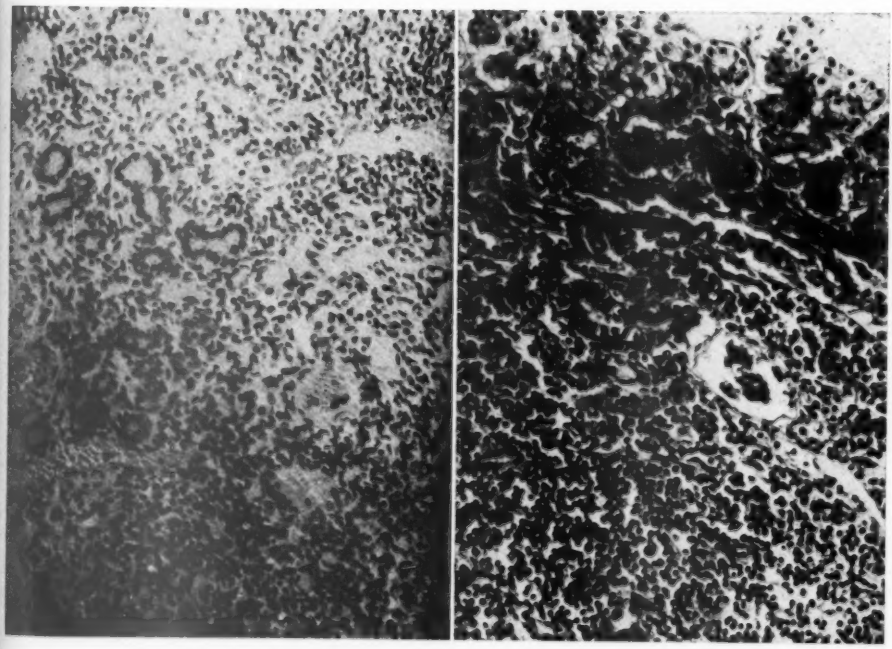

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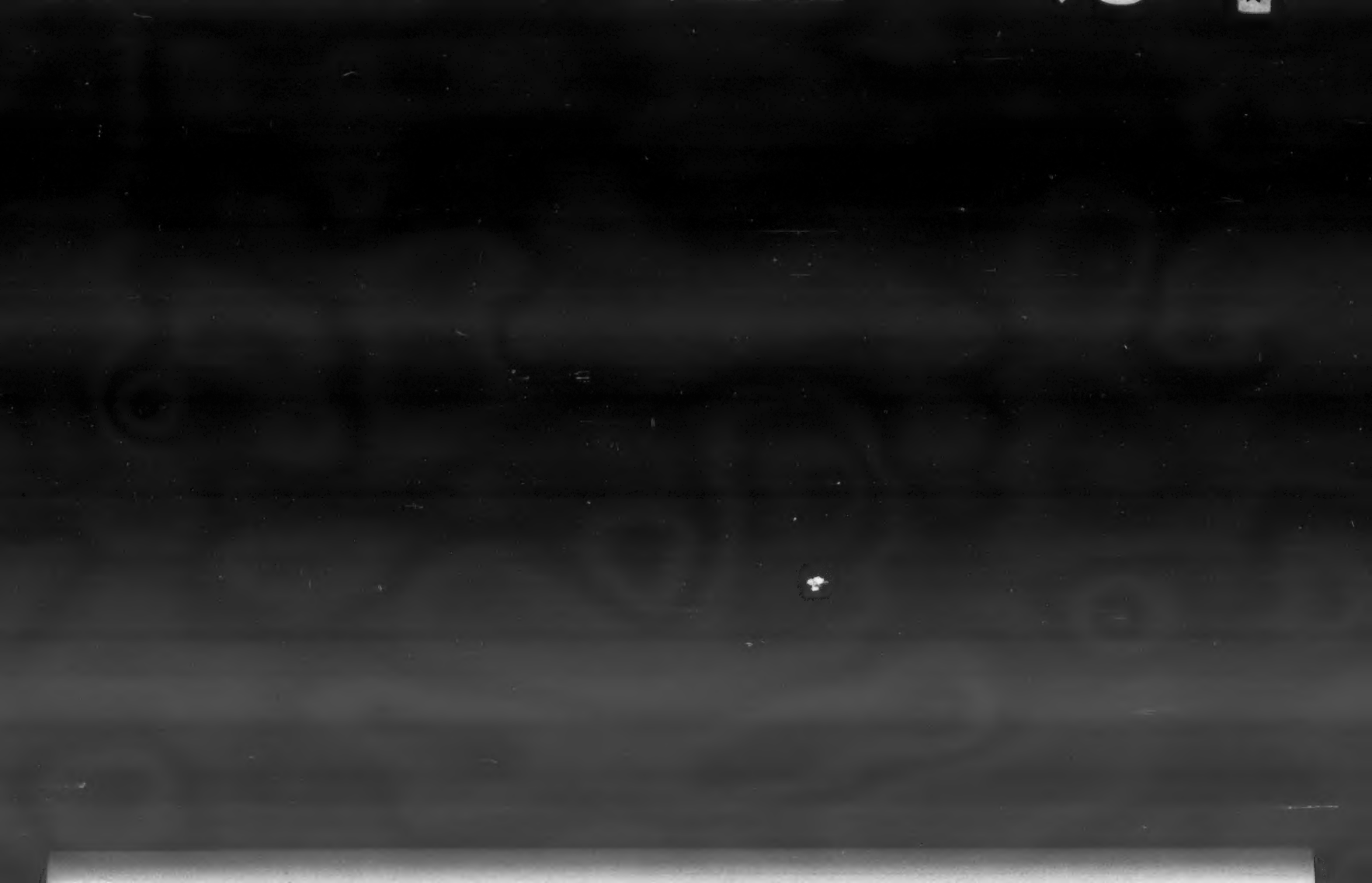
Bryant and Thompson Histogenesis of Papillary Cystadenoma Lymphomatosum

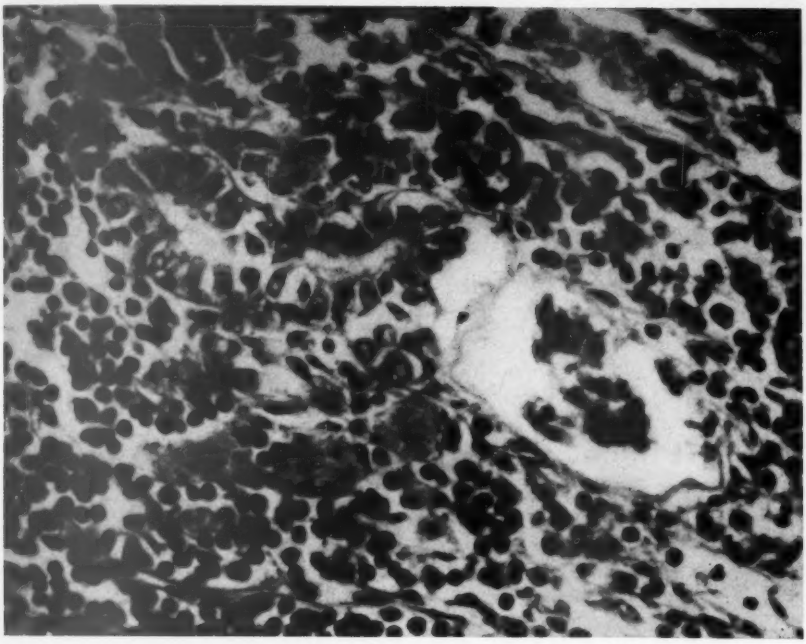
PLATE 114

FIG. 10. Parotid ducts in a lymph node from the parotid gland region of an adult. This is an area from the preceding figure at a higher magnification. Hemalum and eosin stain. × 550.

FIG. 11. A lobule of parotid gland extending into the hilus of a lymph node, from an adult. Hemalum and eosin stain. × 200.

FIG. 12. Parotid ducts showing a transition of the usual type of epithelium to the oxyphilic granular type. Hemalum and eosin stain. × 500.





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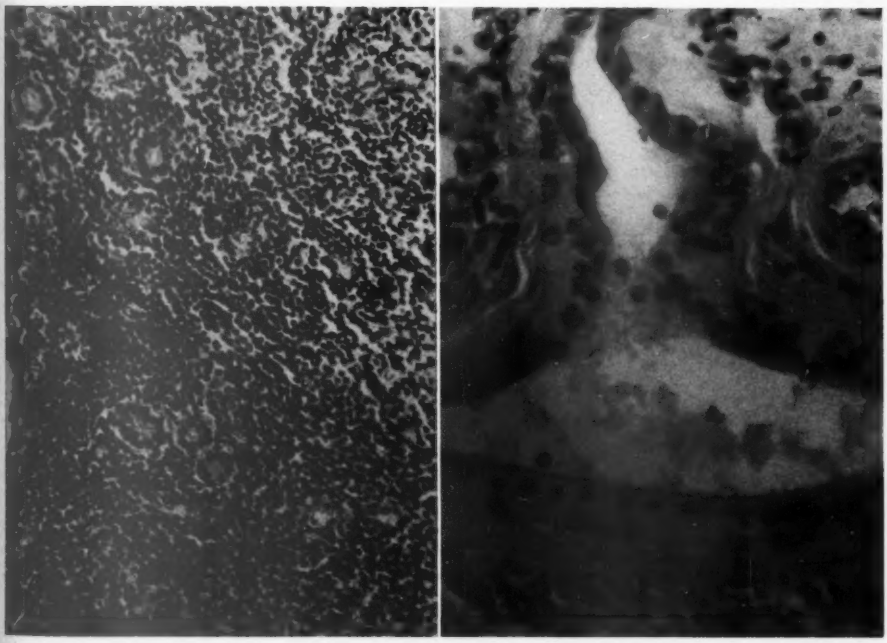

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Bryant and Thompson Histogenesis of Papillary Cystadenoma Lymphomatosum

PLATE 115

FIG. 13. Parotid ducts showing transition of the epithelium to the oxyphilic granular type. Hemalum and eosin stain. × 500.

FIG. 14. Parotid ducts lined by the "clear cell" variant of oxyphilic granular cells. The nuclei of the cells forming the ductal epithelium are located in the luminal one-third of the cell body. In acinar cells of a somewhat similar appearance, the nuclei are located in the basilar one-third of the cell body. Masson's trichrome stain. × 500.

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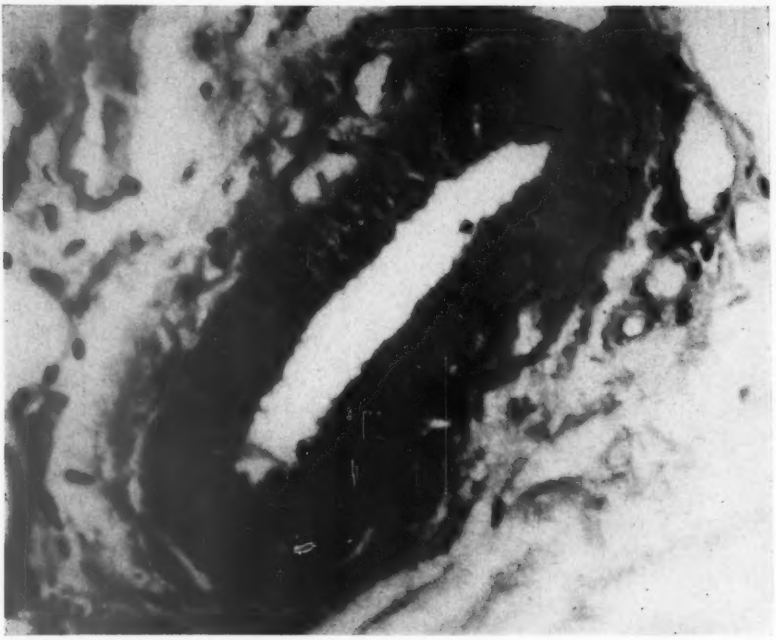

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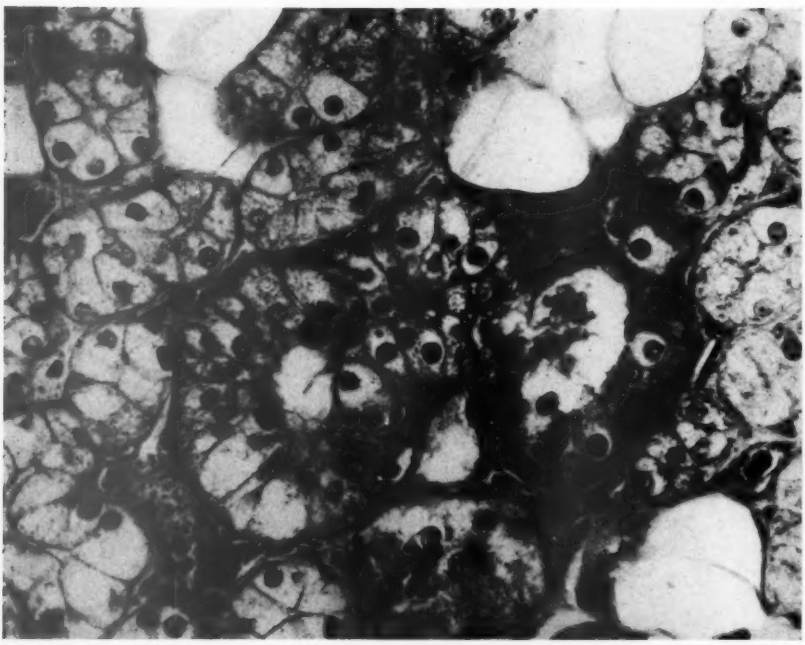
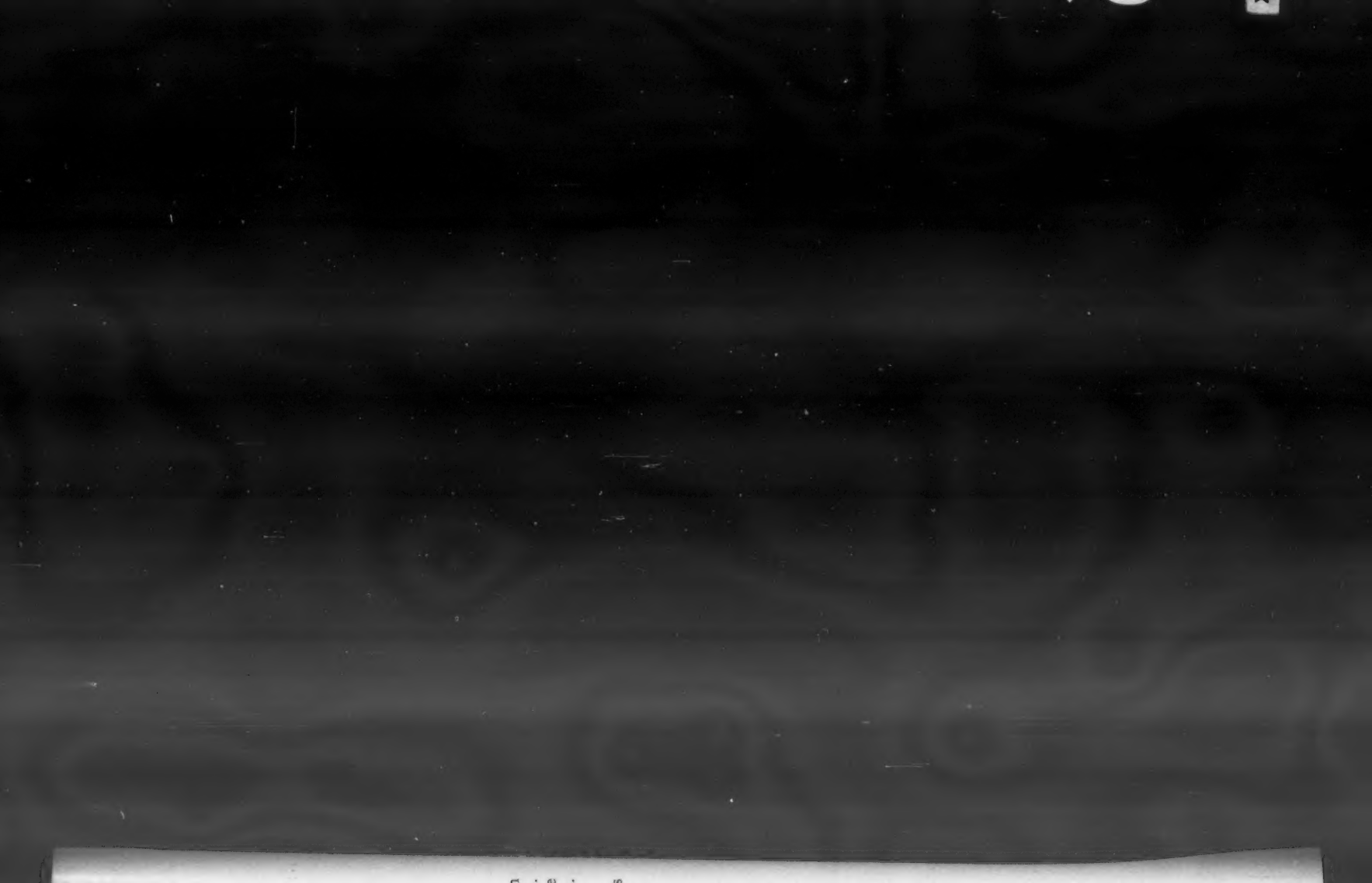


Bryant and Thompson Histogenesis of Papillary Cystadenoma Lymphomatosum

PLATE 116

FIG. 15. A dilated interlobular duct of the submaxillary gland. The epithelium consists of oxyphilic granular cells, and low papillations project into the lumen. The similarity of this epithelium to that of the Warthin tumor and of the oxyphilic granular cell adenoma may be noted. Hemalum and eosin stain. × 200.

FIG. 16. "Clear cells" of the epithelial component of the Warthin tumor. Masson's trichrome stain. × 500.



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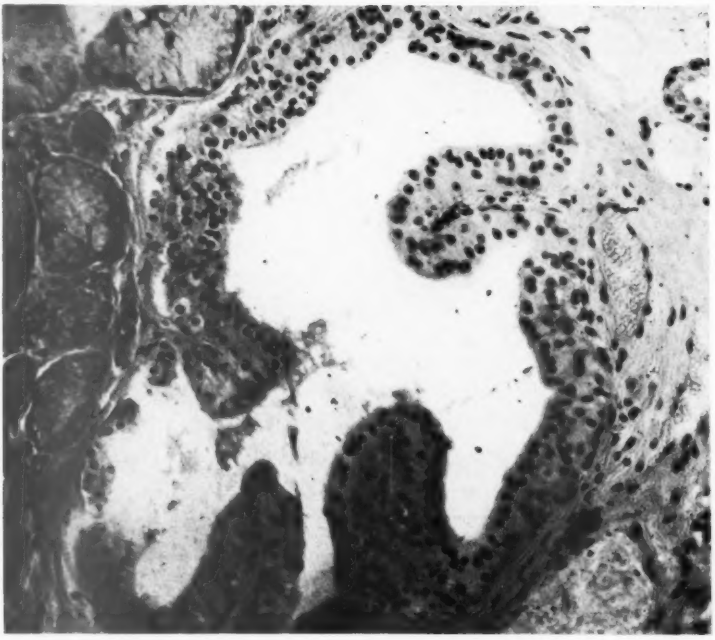

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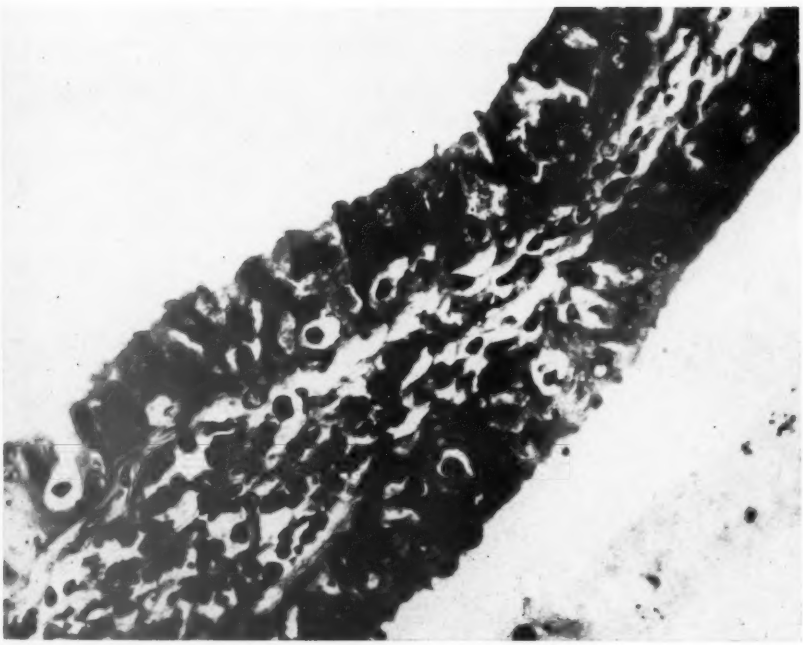

Bryant and Thompson Histogenesis of Papillary Cystadenoma Lymphomatosum

PLATE 117

FIG. 17. Small masses of secretory material may be seen arising from the luminal surface of the epithelium of a Warthin tumor. Masson's trichrome stain. × 500.

FIGS. 18 and 19. Collapsed, sharply delineated cells with pyknotic nuclei among the epithelial cells of a Warthin tumor. These cell bodies are seen forming part of the secretion of these tumors. Masson's trichrome stain. × 500.

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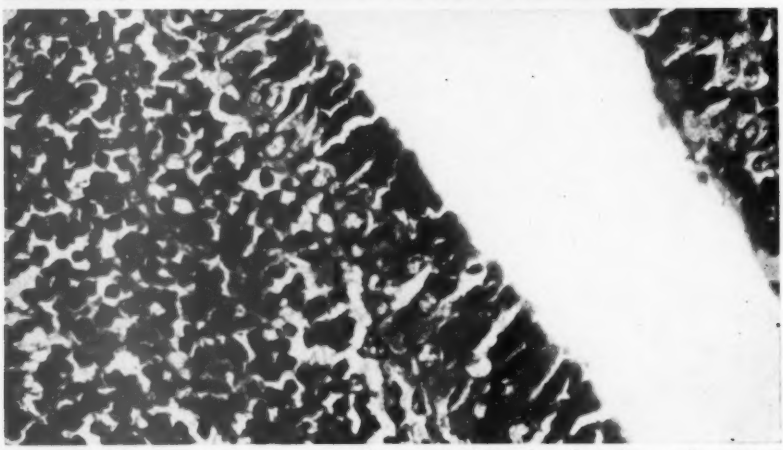
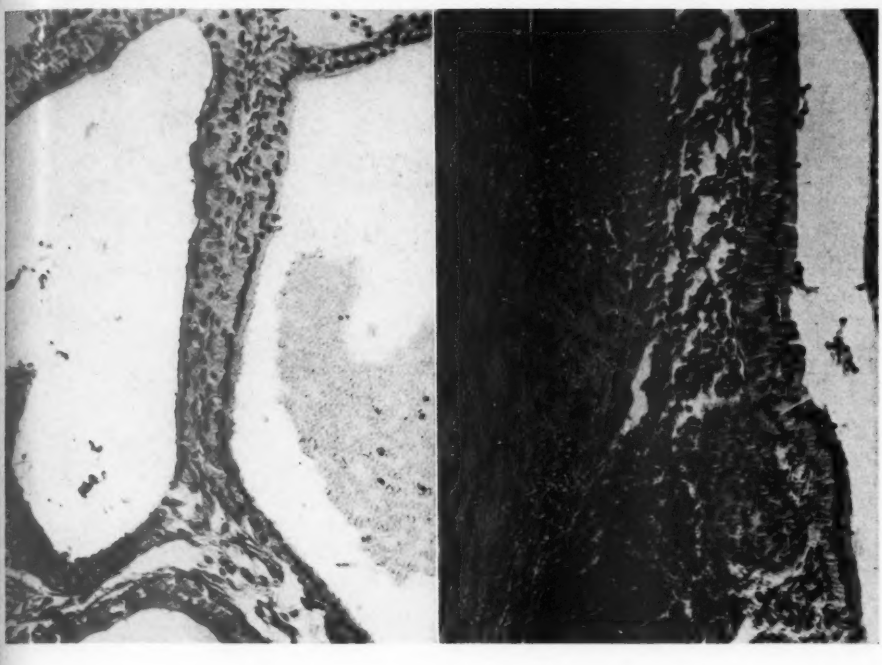


PLATE 118

FIG. 20. An area from a Warthin tumor showing epithelium that compares favorably with that of the usual parotid ductal epithelium (lower portion of the photograph). Stages in the transition to the oxyphilic granular cell type are seen. Hemalum and eosin stain. × 200.

FIG. 21. Oxyphilic granular cells, backed by scar tissue and an inflammatory infiltration, lining cystic spaces of a Warthin tumor. Hemalum and eosin stain. × 200.

FIG. 22. Peripheral lymph sinuses beneath the capsule and extending into the supporting stroma of a Warthin tumor. Hemalum and eosin stain. × 100.



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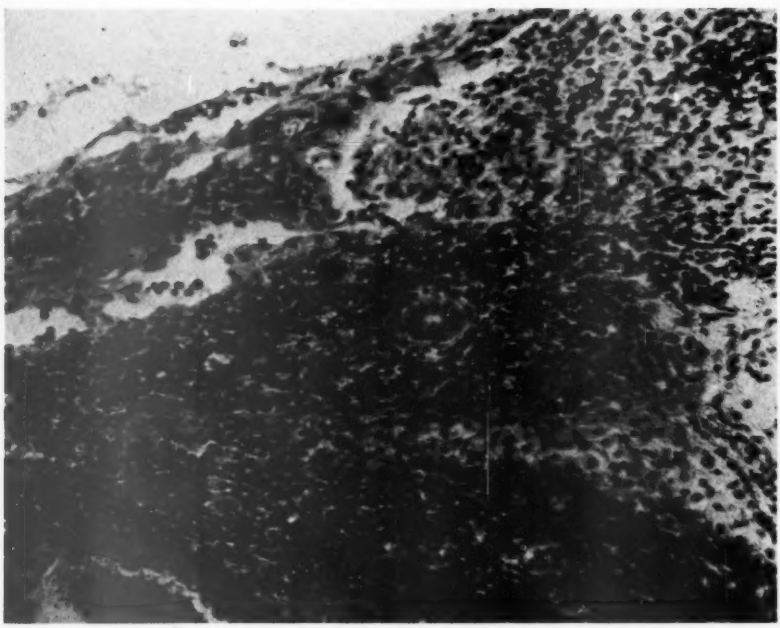

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Bryant and Thompson Histogenesis of Papillary Cystadenoma Lymphomatosum

Plate 119

Fig. 23. A polypoid mass of young scar tissue and associated products of inflammation protruding into a cystic space of a Warthin tumor. Hemalum and eosin stain. × 200.

Fig. 24. A focus of oxyphilic granular cells forming medullary and tubular patterns in a lobule of the parotid gland. Hemalum and eosin stain. × 500.

Fig. 25. A solid epithelial area of a Warthin tumor. Hemalum and eosin stain. × 500.

Fig. 26. A portion of a cystic tumor considered to occupy a position between the simple inclusion of ducts in lymphoid tissue and the Warthin tumor. Hemalum and eosin stain. × 100.

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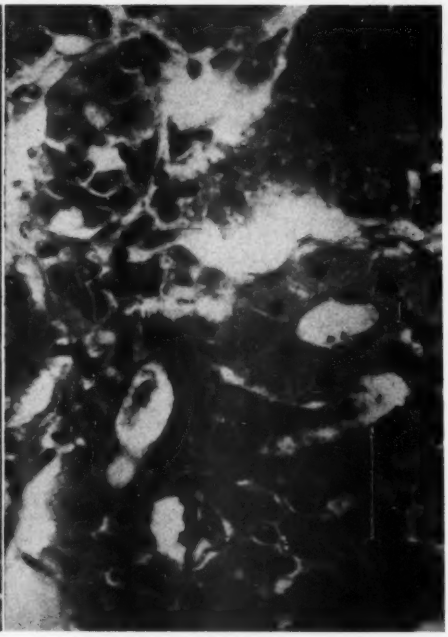

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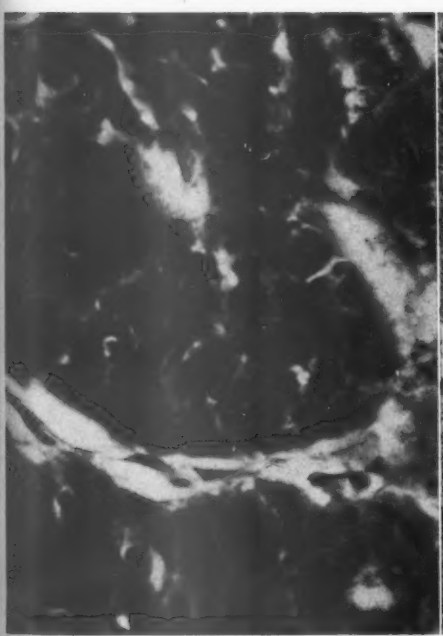

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BILATERAL MAMMARY ARTERITIS

Report of a Case *

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(From the Department of Pathology, Division of Surgical Pathology, McGill University, Montreal, Que.)

During the past decade increasing attention has been attracted to peculiar forms of arteritis that do not appear to fall into the category of any of the more common diseases of arteries. Some of these are apparently regional, while in others autopsy has shown the condition to be generalized. Interest in this field has been stimulated by the extraordinary changes in the blood vessels met with in some patients following sulfa drug therapy and also by the description by Horton, Magath, and Brown[1,2] in 1932 and 1934 of what has come to be termed "temporal arteritis." The case reported here † is presented as one more small piece in the jigsaw puzzle of these conditions, which is being put together gradually.

Report of Case and Pathologic Findings

Mrs. E. McR., a well preserved woman, 64 years of age, was admitted to the Royal Victoria Hospital on November 2, 1947, because of a lump in the right breast. This was not painful or tender and had been first noticed 4 weeks before. She had had typhoid fever in 1905 and phlebitis following pregnancy. In 1946 a glandular polyp of the cervix uteri and a urethral caruncle were removed. At that time her blood pressure was recorded as 165/100 mm. of Hg. To the best of her knowledge she had never taken sulfa drugs.

On admission her temperature, pulse, and respirations were normal. The lump in the right breast was small and hard, somewhat irregular, about 1.3 cm. in diameter, movable, and situated in the upper medial quadrant. Examination of the urine and a blood Wassermann test and determination of the chemical constituents of the blood gave normal or negative findings. The electrocardiogram showed left preponderance with slurring of the QRS deflections, and some tortuosity of the aorta was indicated by roentgenograms of the chest. There were a slight normochromic anemia (84 per cent), a rapid sedimentation velocity of 40 mm. after correction for the volume of packed cells, and a leukocytosis of 8,500 due to increase in polymorphonuclears and monocytes. Such a high sedimentation velocity in a woman apparently not acutely ill is a noteworthy feature. The medical consultant reported mild asymptomatic hypertension (158/96 mm. of Hg), with no evidence of decompensation. Because of the probability that the lesion was malignant, a simple mastectomy was done on November 5, a frozen section at the time having revealed that the lump contained a thickened thrombosed artery and no evidence of carcinoma. There was no demonstrable disease in the other breast.

* Read by title at the Forty-sixth Annual Meeting of The American Association of Pathologists and Bacteriologists, Boston, April 15 and 16, 1949, and presented at the meeting of the Quebec Association of Pathologists, Montreal, May 20, 1949.

Received for publication, September 10, 1949.

† Permission to report this case was granted by Dr. J. C. Armour of the Department of Surgery, Royal Victoria Hospital, Montreal.

The breast was composed of abundant fat with small amounts of fibrous appearing parenchyma. There were no areas of induration in it, as the lump had been removed for frozen section. This small mass consisted of firmer fat and through it coursed a thickened blood vessel which appeared to be occluded by a thrombus.

Histologic sections of the small areas of breast tissue in the abundant fat of the larger specimen showed typical fibrous atrophic parenchyma with moderately dilated ducts. An occasional lobule was preserved and some showed hyperplastic changes in the epithelial elements. The vascular channels were not remarkable. Sections of the mass showed it to consist of a markedly thickened artery with surrounding infiltrated fat tissue (Fig. 1). The artery was of medium caliber and from its position and size it was a perforating branch of the internal mammary, which supplies the medial portion of the gland. The surrounding tissue for a considerable distance showed proliferation of the fixed tissue elements with separation of the fat cells. This became gradually more marked as one approached the adventitia until a fairly solid fibrous mantle was produced. About the vasa vasorum were clusters of lymphocytes with an occasional eosinophil. Bands of fibroblastic cells spread outward in spider-like fashion through the fat. The adventitia was markedly widened and its outer boundary poorly defined. It consisted of proliferating fibroblasts between collagen fibrils, swollen edematous endothelial elements, and occasional lymphocytes. The external elastic lamina was thin and fragmented, and in the media the inflammatory changes became more active and granulomatous. This whole layer was replaced by a loosely arranged proliferating meshwork of fixed tissue elements in which were found lymphocytes, plasma cells, an occasional neutrophil and eosinophil, epithelioid cells, and multinucleated giant cells. The last were not numerous, showed several vesicular nuclei, and appeared to arise from lack of separation of the proliferating fixed tissue elements (Fig. 2). The internal elastic lamina had undergone extraordinary alteration. In some areas it was merely thin and fragmented, but from this was transformed gradually into tremendously thickened masses of amorphous material. These apparently consisted of degenerated elastic tissue and appeared to have undergone some calcification. The fibers first thickened throughout their whole length, then nodules appeared along their course, particularly at the ends, with final breakup and loss of continuity. No foreign body giant cells had developed about the masses. Fine elastic fibrils spread into the intima. This inner layer was quite thin and relatively well preserved. It showed some edematous swelling, slight exudate, and loss of the endothelial lining

cells. Beginning organization of the recent thrombus in the lumen was taking place. No other artery in the gland was similarly affected.

On January 9, approximately 2 months later, this patient presented herself for routine check-up, complaining of pain in the left breast. During the interval she had been quite well and believed that she had gained strength. She was readmitted to the hospital on January 15. At that time her temperature, pulse, and respirations were normal. The blood pressure was 155/95 mm. of Hg. The scar of the recent right mastectomy was well healed. A lump now present in the left breast was situated medial to the nipple, measured 3 by 2 cm., was tender, firm, and fixed to the skin. Clinically, it appeared to be a carcinoma. On January 16 a radial elliptical incision was made to include the tumor mass. Frozen sections revealed a lesion similar to that found in the other breast, *i.e.*, arteritis involving one of the penetrating branches of the internal mammary artery. The patient made an uneventful recovery and was discharged on the fifth day.

Up to the time of this report, a period of 16 months since leaving the hospital, she has remained well. On December 29, 1948, a hematologic examination was carried out. The blood picture was similar to that at the time of her first operation. It showed a slight degree of hypochromic anemia, markedly increased sedimentation velocity, and leukocytosis of 8,900 due to increase in polymorphonuclears and monocytes with slight rise in eosinophils. The persistence of the high sedimentation rate is the most noteworthy feature. To what extent it may be interpreted as evidence of a progressing and generalizing arterial disease in the absence of symptoms is difficult to state.

The surgical specimen from the left breast consisted of a mass of fibro-fatty tissue measuring 11 by 7 cm. and covered by an ellipse of skin 8 by 2.5 cm. Near one border of the skin and beneath a point where it was puckered and indrawn there was a small nodule which appeared to be indurated fat, 1 cm. in diameter. This had been incised to reveal a centrally placed thick-walled and thrombosed vessel, about which there was a slight reddish blue discoloration.

Sections of the little remaining breast parenchyma showed it to be atrophic, involuted, and fibrous, with slightly dilated ducts. Here the vascular channels were free. Sections of the mass showed fatty tissue in which were three small arteries, apparently branches of a perforating division of the internal mammary. In one of these there was a simple calcareous arteriosclerotic lesion of the media but no exudate, while the other two presented inflammatory changes similar to those in the right breast 2 months previously. The following points of difference, however, should be noted. In one of these two branches the lesion appeared of longer standing (Fig. 3). The lumen was obliterated by organization of the thrombus and several giant cells of foreign body type lay adjacent to the masses of degenerated elastic fibers. These fibers appeared to form a palisade of parallel strands before fusing into the amorphous mass (Fig. 4). Many fragmented elastic fibers were found in the bands of fibrous tissue which extended into the fat. The other branch of the

artery was markedly dilated, as if to compensate for the occlusion of the first, and the thrombus in its lumen was very recent with signs of organization just beginning. It seems that the occlusion of this vessel led to pain and thus brought the lump to the attention of the patient. In the proliferating fibroblastic tissue of the fat about this vessel there were many lipid-rich cells not encountered elsewhere. In general, however, the character of the granulomatous reaction in the various coats of these arteries was essentially the same as that described in the other breast.

Discussion

From the pathologic standpoint the principal problems that arise for discussion in this case are concerned with the etiology, pathogenesis, and classification of the arterial lesion. In regard to the cause, no positive conclusions are possible. Cultures were not taken from the tissues at the time of the operations, but experience is strongly against any probability that the results would have been informative. Search of the histologic sections for bacteria by various staining methods was without results.

The pathologic changes in the various arteries leads to the conclusion that this is a subacute inflammatory process having certain peculiar degenerative and granulomatous features. The impression is gained that the oldest and initial lesion is in the adventitia. Here one finds lymphocytes about the vasa vasorum, which remain patent, and an extensive fibroblastic proliferation spreading out into the fat. Subsequently the media becomes involved by regressive changes in its fibrillar structure. Fragmentation and necrosis occur and are followed by a granulomatous inflammatory reaction of the fixed tissue elements. Lymphocytes, plasma cells, occasional eosinophils, epithelioid cells, and giant cells lie in a fibroblastic meshwork. At the same time an extraordinary change is taking place in the internal elastic lamina. Thickening, fragmentation, beading, and then palisade arrangement of the fibers occur, with fusion into amorphous masses. Foreign body giant cells may form about the clumps. The intima remains relatively free except for a few lymphocytes and apparent infiltration of newly formed elastic fibrils. There is no evidence of fibrinoid necrosis. Thrombosis is a late event but may be followed by organization of the clot and recanalization. The process appears to be confined to a relatively small segment of the artery in the breast, but one cannot exclude the possibility that it may have extended inward.

As to precise diagnosis and classification of this arterial lesion, there are numerous difficulties. Such are bound to arise whenever definite etiologic factors are unknown, and for classification one is forced to

depend upon the clinical course and pathologic changes for the recognition of a disease entity. In this case arteritis affected the penetrating branches of the internal mammary arteries of both breasts in an elderly woman. Except for the persistence of an increased sedimentation velocity and mild leukocytosis, there was nothing after a period of 16 months to indicate that the lesion was progressive or had generalized. The inflammatory reaction in the arteries had certain granulomatous features, displayed extraordinary regressive changes in the internal elastic lamina, and eventually caused thrombosis.

In attempting to classify this condition, many forms of both relatively common and of rarer arterial disease may at once be discarded on the grounds that they are not inflammatory or, if so, display a distinct etiologic factor such as syphilis or tuberculosis. There remains for consideration, however, a group of idiopathic inflammatory diseases such as periarteritis nodosa, thromboangiitis obliterans, and temporal arteritis which demands special consideration. The giant cell arteritis described by Gilmour[3] is simply generalized temporal arteritis under another name and the term has not come into common use as it is believed that the multinucleated cells are not the pathognomonic feature. One of the 4 cases Gilmour described was probably tuberculosis. The rare forms of so-called disseminated arteritis and the arteritis associated with lupus erythematosus differ so fundamentally from this lesion that they do not merit consideration.

From the clinical standpoint this case simulates temporal arteritis most closely. That disease occurs predominately in women over 55 years of age, frequently with hypertension, who show leukocytosis and a high sedimentation rate and are usually febrile. In most cases the disease is self-limiting and recovery is accelerated by removing the involved vessel. However, generalizing and fatal forms have been described. Thromboangiitis obliterans occurs almost entirely in men under 50 years of age, and is primarily a disease of the vessels of the extremities. Periarteritis nodosa is most common in young men, involves the visceral, not peripheral, vessels, is usually accompanied by eosinophilia, and is fatal within 1 year.

Also, from the standpoint of the histopathologic changes in the involved vessels, the lesion conforms most closely to those occurring in temporal arteritis. Most authors[1-5] writing on this subject have stressed the fibrillar degeneration and necrosis of the media with replacement by granulomatous inflammatory tissue, the profound disturbance in the internal elastic lamina with palisade formation of fibrils and fusion into amorphous masses, and the relative freedom of the intima except for the eventual thrombosis of the lumen and organization.

This picture, which has come to be more or less pathognomonic for temporal arteritis, is imitated precisely in my case. In periarteritis nodosa the initial lesion is more acute and necrosis is associated with cellular exudate. Infiltration of the vessel wall with polymorphonuclear neutrophils, lymphocytes, and often eosinophils is marked. Aneurysms occur and there is a tendency to involve the smaller arterioles. In thromboangiitis obliterans we are dealing with an inflammatory, non-suppurative periarteritis and/or phlebitis. Thrombosis occurs early and without necrosis of the wall of the vessel. The lumen is often small owing to contraction. Endothelial proliferation and fibroblastic organization of the clot are noteworthy in a surprisingly well preserved vessel.[6-8]

It would appear, therefore, both from the clinical and histopathologic standpoints, that the process in my case approaches most closely that which has been recognized as affecting the temporal arteries and has come to be designated "temporal arteritis." Several cases[3-5,9,10] have been described in which other arteries were involved, such as the carotid, subclavian, innominate, and even the renal, iliacs, and coronaries, and the aorta. There seems no reason, therefore, why the penetrating branches of the internal mammary arteries could not be the seat of the lesion. No reference, however, to such involvement could be found in the literature.

When one comes to the question of giving a name to this condition, the same difficulty which has been pointed out in respect to the use of the term "temporal arteritis" arises. With knowledge that this disease may generalize, a name designating a particular artery was no longer satisfactory. Gilmour,[3] as mentioned previously, attempted to avoid it by the term "giant cell arteritis." However, it is generally thought that until an etiologic factor is discovered, or a more satisfactory specific term descriptive of the characteristic histopathologic changes is obtained, the present terminology should be retained. Under such circumstances there is no choice but to designate the condition described in this paper as bilateral mammary arteritis.

Conclusions

A case is reported of a woman, 63 years of age, who within a period of 2 months developed lumps in both breasts as the result of an inflammatory thrombosing condition of the penetrating branches of the internal mammary arteries. The clinical features of the case and the histopathologic changes in the vessels correspond most closely to those met with in temporal arteritis. No recurrence or evidence of generaliza-

tion of the disease was apparent 16 months after surgical removal of the lesions.

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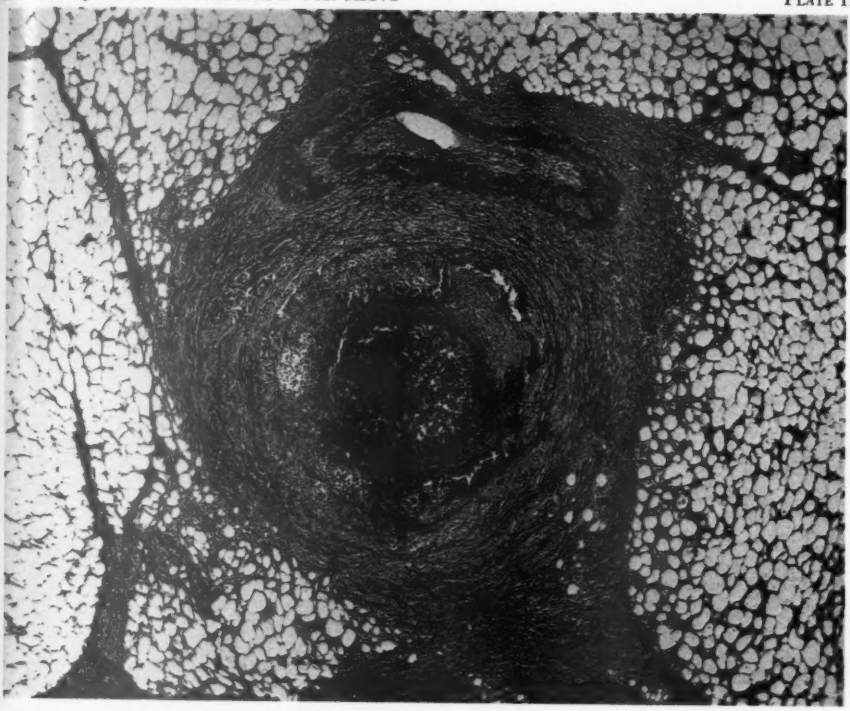
[*Illustrations follow*]

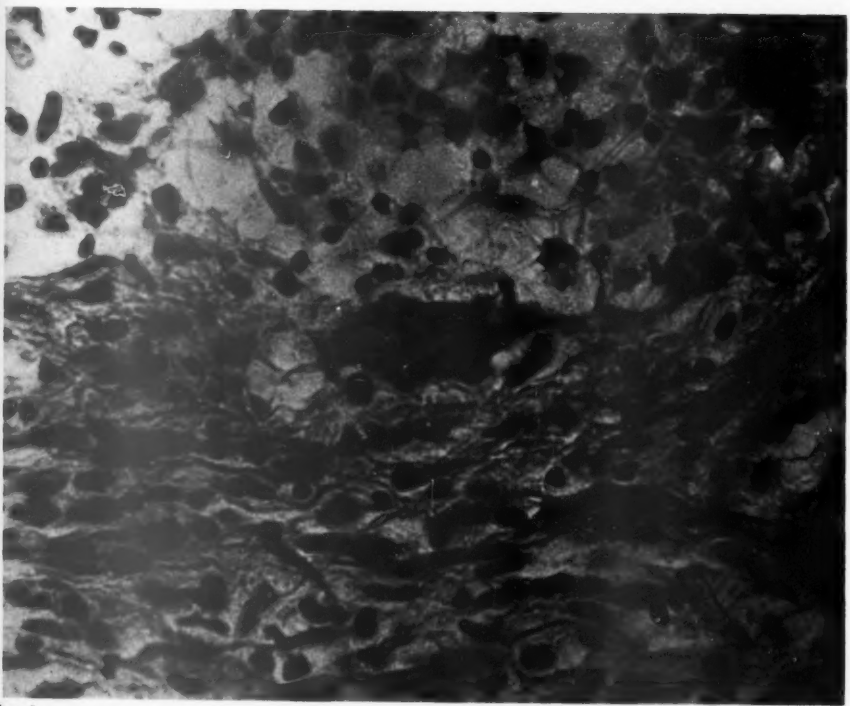
DESCRIPTION OF PLATES

PLATE 120

FIG. 1. The arterial lesion in the right breast, with the proliferating inflammatory process extending into the surrounding adipose tissue.

FIG. 2. Right breast. At a higher magnification the character of the granulomatous reaction in the media of the artery can be seen.



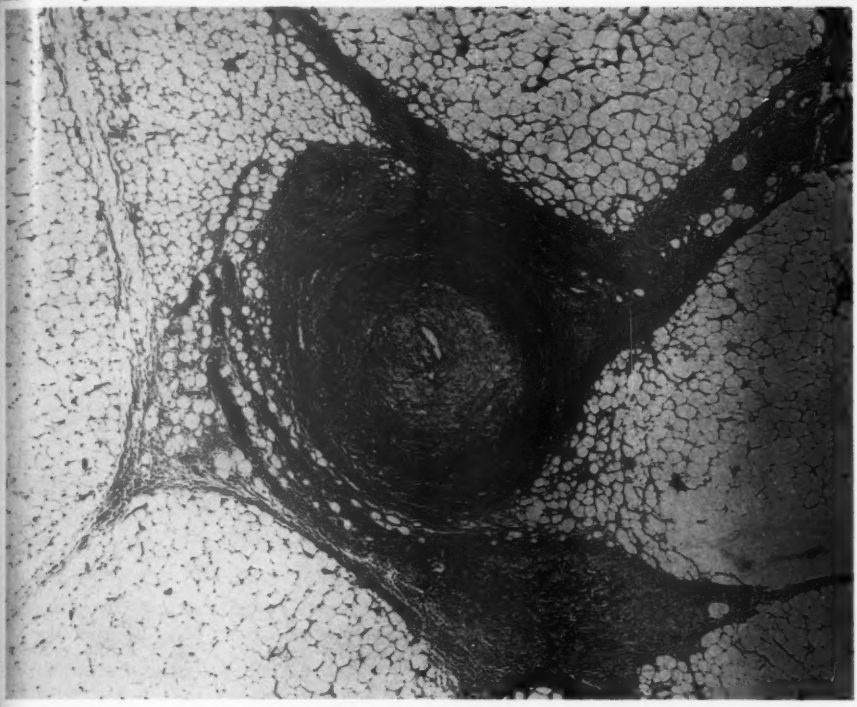


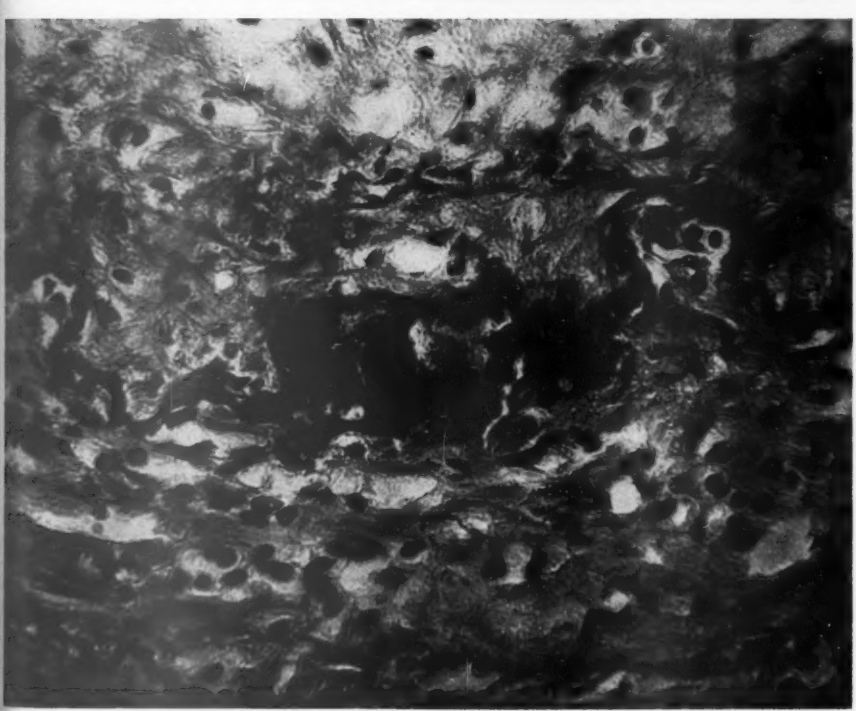
Waugh Bilateral Mammary Arteritis

PLATE 121

FIG. 3. Left breast. The lesion of a perforating division of the internal mammary artery shows an older productive process than that shown in Figure 1.

FIG. 4. Left breast. At a higher magnification the aggregation of necrotic hyaline material into amorphous masses can be seen as well as an arrangement of fibrils in a palisade formation.





A HISTOLOGIC STUDY OF MUSCLES AND NERVES IN POLIOMYELITIS *

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The pathologic changes in voluntary muscles and nerves in poliomyelitis have received little attention in comparison with the lesions in the central nervous system. Extensive biopsy studies were made by Kopits[1] in cases of several years' duration, and by Hipps[2] who correlated anatomical features with muscular function and clinical treatment. Hassin[3] and Horányi-Hechst[4] found muscle alterations in acute cases, and Flynn[5] reported advanced degeneration in many muscles of a patient who died 5½ months after the onset of the disease. Clawson, Noble, and Lufkin[6] examined samples of seven muscles from 22 cases of acute poliomyelitis and found areas of focal myositis in 2 patients. Important contributions in this field include also those of Carey, Massopust, Zeit, and Haushalter,[7] who found disintegration of the motor end-plates of human beings and monkeys in the earliest stages of the disease. Dublin, Bede, and Brown[8] observed degeneration of the myoneural junctions, nerve fibers, and muscles in biopsy specimens from 3 patients who had had poliomyelitis for from 5 to 7 weeks.

Muscles and nerves were obtained at autopsy from 12 patients during the poliomyelitis epidemic in Colorado in 1946 and subsequently. Five deaths occurred during the acute stage from the third to seventh day after the onset of clinical symptoms. Three patients died from 16 to 60 days after the onset. Four patients who were chronically ill survived from 14 months to 3 years after the initial paralysis.

Samples of the following muscles, with some exceptions and additions, were taken routinely: temporal, sternocleidomastoid, pectoralis major, deltoid, biceps brachii, intercostals, diaphragm, rectus abdominis, psoas, quadriceps femoris, adductor magnus, and gastrocnemius. The nerves that were studied included the phrenic, intercostals, the three cords of the brachial plexus, femoral, and sciatic. These muscles and nerves presented a characteristic histologic picture independent of their location. Other sites were not examined in order to avoid mutilation.

METHODS

Specimens of the muscles were fixed in both neutral formalin and Zenker's solution. The stains employed on paraffin-embedded material

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were hematoxylin and eosin, and van Gieson's. The scharlach-R stain and occasionally the von Braunmühl silver stain[9] were applied to frozen sections. The nerves were fixed in neutral formalin and routinely stained by the Spielmeyer, scharlach-R, and hematoxylin and eosin methods. In addition, the van Gieson, Bodian, von Braunmühl, and Weil stains were used when indicated. Carey's[10] method of gold impregnation for nerve endings was soon abandoned because the tissue could not be obtained soon enough after death to provide reliable results; however, the autopsies were performed from 6 to 12 hours post mortem, and autolytic changes were otherwise negligible.

Report of Cases

Case 1

A white girl, 17 years of age, died 3 days after the onset of poliomyelitis. Circulatory collapse dominated the clinical picture, and muscular paralysis was not demonstrated. Histologic lesions were widespread in the brain stem, but most of the motor nerve cells of the spinal cord appeared normal. The majority of the muscle fibers were normal in size and revealed prominent cross striations. In hematoxylin and eosin preparations, long segments of numerous fibers stained yellow or gray instead of red. In frozen sections treated with the Braunmühl stain, some of the fibers were peppered diffusely with black granules; as a rule, however, the fibers were normal and argentophilic granules sharply outlined the anisotropic bands. A minimal degree of fatty degeneration involved occasional fibers of the diaphragm and intercostal muscles. Occasional myelinated *nerve* fibers showed irregular swelling and appeared foamy or vacuolated. Neutral fat was not present. The axis cylinders showed slightly reduced staining qualities.

Case 2

A white male, 20 years of age, died on the third day after the onset of poliomyelitis within an hour of admission to the hospital. Acute poliomyelitic lesions were severe in the medulla and spinal cord. In the *muscles,* a few small areas showed proliferation of hypolemmal nuclei. More impressive was the presence of abundant fine lipoid granules in numerous fibers of all muscles examined. The *nerves,* and the femoral nerve in particular, showed striking stripes of demyelination of varying width. Much of the remaining myelin was beaded and irregularly stained. Only a few isolated lipoid granules were present. The staining affinity of the axis cylinders was reduced; some were irregularly swollen and exhibited increased argentophilia; in some fields stained axons were not seen. Endoneural fibers were diffusely hyperplastic.

Case 3

A white youth, 17 years of age, died of respiratory failure 4 days following the onset of bulbar poliomyelitis. The medulla showed extensive neuronal destruction and gliosis, and the spinal cord revealed damaged anterior horn cells in scattered areas. Some fibers of most *muscles* showed slight swelling, longitudinal splitting apart of the myofibrils, and slight focal proliferations of peripheral sarcolemmal nuclei. The cross striations were prominent. A few fibers of intercostal muscle displayed necrosis and numerous sarcolemmal nuclei mixed with the débris. Early fatty metamorphosis was present in scattered fibers of each muscle, especially in those of the tongue and the diaphragm. Alternating *nerve* fibers and small patches of fibers were completely demyelinated (Fig. 5). In some nerve bundles the myelin was swollen, beaded, or vacuolated. Neutral fat was not present. Several small collagenous scars and proliferations of endoneural fibroblasts were present in the nerve trunks. The axis cylinders appeared normal.

Case 4

A white boy, 9 years of age, had poliomyelitis of the bulbo-spinal type. He died on the fourth day after the development of paralysis of the muscles of respiration and of the extremities. The *muscle* fibers took the hematoxylin and eosin stain unevenly: some stained dark red, others light gray. The cross striations were distinct, and the number of sarcolemmal nuclei was not greatly increased. Early fatty metamorphosis was an impressive finding in each muscle. The majority of the muscle fibers contained abundant, evenly distributed fat granules. Normal fibers were intermingled with fatty ones. The *nerves* were not available for examination.

Case 5

A white man, 27 years of age, died 7 days after the onset of poliomyelitis of bulbar type. About 24 hours before death, weakness was noted in the right arm. The motor cortex, the medulla, and the spinal cord at all levels showed severe poliomyelitic changes. The *muscle* fibers were normal in size, and most of them possessed distinct cross and longitudinal striations. Scattered fibers, most numerous in the right biceps brachii muscle and the diaphragm, failed to take the hematoxylin and eosin stain normally; they were either yellow or gray and homogeneous (Fig. 1). Focal areas of the fibers were necrotic, and a few leukocytes were mixed with the clumped débris of the contractile substance. In other foci, the fiber substance was pale or absent, and the sarcolemmal sheath was collapsed. There were also swelling, fibrillary splitting, and

a slight proliferation of sarcolemmal nuclei. Minute globules of fat were seen within isolated muscle fibers. In the *nerves*, the myelin was beaded and stained poorly in patchy areas. Neutral fat was not detected. Moderate numbers of the axis cylinders showed irregular swelling, fragmentation, and paling.

Case 6

A white girl, 18 years of age, developed bulbo-spinal poliomyelitis and died 16 days later. Both hands, the left arm and shoulder, the respiratory muscles, and both lower extremities were paralyzed. Patchy collapse of the lungs and bronchopneumonia contributed to her death. Degeneration of the medulla was advanced, and the motor nerve cells in the cervical and lumbar portions of the spinal cord were almost totally destroyed. The *muscle* fibers were swollen, crowded together, and showed prominent fibrillary splitting (Fig. 7). Occasional segments were dark red and homogeneous, but on the whole staining was uniform, and cross striations were present although pale. The sarcolemmal nuclei were increased in numbers, rather heavily in some areas. They were oval, spindle-shaped, or rod-like; some were pyknotic or fragmented. Fatty metamorphosis was the principal histologic change, and was advanced in every muscle (Fig. 2). The anisotropic bands were accentuated by closely packed, fairly uniform, minute fat droplets. One or more affected fibers alternated with normal fibers. A coarse scattering of argentophilic granules, unrelated to the cross striations, was observed in some areas (Fig. 8). A few small collections of lymphocytes and plasma cells were present in the perivascular tissue, and there was a proliferation of endothelial and adventitial cells of the small blood vessels. Large patches of *nerve* fibers were extensively demyelinated, and some axis cylinders had been destroyed. Globules of neutral fat were present in the degenerated fibers in both the nerve trunks and in the small intramuscular branches of the nerves (Fig. 3). The large nerves showed occasional interstitial infiltrations of lymphocytes.

Case 7

A white man, 27 years of age, died of massive pulmonary embolism secondary to thrombosis of the deep veins of the legs 23 days after the onset of poliomyelitis of the bulbo-spinal type. By the time of death he had regained much strength in the paralyzed larynx, the diaphragm and intercostal muscles, and the lower extremities. Poliomyelitic lesions in the medulla and spinal cord were minimal. The *muscle* fibers stained uniformly and there were distinct cross and longitudinal striations. The fibers were swollen and packed together in many places, and showed a

slight focal increase in the number of sarcolemmal nuclei. Demyelination was extensive only in the intercostal *nerves*, and occasional fibers showed fatty degeneration; small patchy and linear hyaline scars were present (Fig. 9). The axis cylinders were normal except for occasional swellings.

Case 8

A white man, 28 years of age, had poliomyelitis of the bulbo-spinal type followed by wasting of all the skeletal muscles. He appeared to be improving when he died suddenly 60 days after the onset of the disease. Two weeks previously he had predicted, almost to the hour, the time of his death. The autopsy failed to reveal the immediate cause of death. The medulla presented only slight histologic evidence of poliomyelitis, but most motor nerve cells had disappeared from the spinal cord at all levels. Many *muscles* contained atrophic fibers, which were usually intermingled with slightly swollen fibers. Many dark wavy fibers were about one-third or less the normal diameter (Fig. 10). Focal rarefaction and disintegration of the muscle-fiber substance were not uncommon, and moderate to severe diffuse proliferation of sarcolemmal nuclei was present. The respiratory muscles contained some fibers which were filled with coarse disordered fat droplets. Cross striations usually were seen in preparations treated with the silver stain, but they were often indistinct in the hematoxylin and eosin sections. In the *nerves* there were focal scarring, edema, and extensive demyelination. Lipoid material was present in quantity only in the branches of the intercostal nerves.

Case 9

A white man, 42 years of age, died of collapse of the lungs 14 months after the onset of poliomyelitis of the bulbo-spinal type. He had developed quadriplegia. The medulla showed minimal microscopic changes. The anterior horns in the lumbar portion of the spinal cord were replaced by cystic cavities, and elsewhere they showed gliosis with reduced numbers of motor cells. The anterior and lateral columns exhibited advanced demyelination and heavy infiltration with gitter cells. The *muscles* showed all degrees of atrophy. In many of the fascicles, all fibers were extremely narrow, but in others there was intermixture of normal and hypertrophic fibers with the degenerated fibers. Atrophic and hypertrophic fibers were equally numerous in the respiratory muscles. Often the enlarged fibers were dark red, sometimes without cross striations, and frequently they showed longitudinal fission or contained circumscribed, pale, finely granular areas or vacuoles (Fig. 12). An occasional fiber had undergone coagulation necrosis and infiltration

by sarcolemmal nuclei, lymphocytes, and a few polymorphonuclear leukocytes (Fig. 11). Dense clusters of sarcolemmal nuclei were located within the sarcolemmal sheaths in small areas where the muscle-fiber substance had disappeared. Proliferated nuclei also surrounded muscle fragments (Fig. 4). Small droplets of neutral fat clustered about the plasmodia. Some of the narrowest fibers were uniform in width, showed no fragmentation, and exhibited prominent cross striations. Other fibers of similar width were pale and without internal structure. So-called "Ringbinden" were found occasionally, but their pathologic significance was doubtful as they occur in normal muscle.[11] Rare foci of fatty degeneration were noted in fibers of normal width. The muscle spindle sheaths were expanded. Fibrous scars and small islands of adipose tissue were present throughout the muscles. Extensive demyelination was present in uneven distribution in most *nerves*. Conspicuous scars were found. A large collection of lymphocytes was present in the posterior cord of the brachial plexus. Lipoid changes were absent.

Case 10

A white woman, 33 years of age, died 16 months after the onset of poliomyelitis of the bulbo-spinal type. She had been in a respirator continuously. Collapse of the lungs and a pulmonary infarct caused death. The brain was not available. The anterior horns of the spinal cord contained rare motor nerve cells and massive infiltrates of protoplasmic astrocytes and microglia. The anterior and lateral columns showed advanced secondary degeneration and infiltration with many gitter cells. Degeneration was advanced in all *muscles*. The fibers were moderately to severely atrophic; some were shrunken away from the sarcolemma, fragmented, and hyperacidophilic; others were pale, vacuolated, or showed fibrillary splitting. Cross striations were visible in paraffin or frozen sections in some of the narrowest fibers. In several muscles plasmodia were exceptionally numerous, consisting of as many as twenty or more sarcolemmal nuclei, some of which were pyknotic. There were rare minute hemorrhages and focal necroses of muscle fibers. Fatty degeneration was not seen. Dense fibrous scarring was prominent in some locations, as the intercostal muscles, and a widespread proliferation of collagen fibrils between muscle fibers was present in other areas. Masses of adipose tissue infiltrated the muscles. Both minute and large interstitial collections of lymphocytes were numerous (Fig. 14). Patchy demyelination and fibrosis were present in all *nerves* (Fig. 6). However, the intensity of changes was much less than that in the

muscles. Axis cylinders were pale or absent in a few sheaths and swollen in others.

Case 11

A white boy, 8 years of age, survived 32 months after the onset of bulbo-spinal poliomyelitis. He had paresis of the respiratory muscles and progressive paralysis of the extremities. He had unexplained episodes of cyanosis and coma terminally. Gliosis was prominent in the reticular substance of the medulla, and on an average only three or four motor cells were present in each anterior horn at all levels of the spinal cord. In the *muscles* an impressive combination of extreme atrophy and hypertrophy was present. The fibers of the abdominal muscles were hypervoluminous; the gastrocnemius showed masses of hypertrophic fibers that interdigitated with bundles of extremely shrunken ones (Fig. 15); isolated enlarged fibers were observed in the atrophic pectoral muscle (Fig. 13). In the hypertrophic muscles fibrillary splitting was pronounced, and the myofibrils of one fiber blended so closely with those adjacent that the sarcolemmal sheaths were indistinguishable. The degenerated fibers were wavy, narrow, frequently hyperacidophilic, and fragmented. A moderate proliferation of sarcolemmal nuclei was observed, but plasmodia were not numerous. The nuclei were round in some areas and oval, pale, and vesicular in others; some were dark and flattened. The muscle-spindle capsules were dilated. Dense masses of collagen incased, blended with, and replaced both the atrophic and normal fibers of the diaphragm and intercostal muscles. Varying amounts of adipose tissue and a few lymphocytes were present diffusely in the connective tissue. Only the *nerves* of the brachial plexus were examined. Numerous scattered fibers were devoid of myelin, and others exhibited foamy myelin. The axis cylinders failed to show remarkable change.

Case 12

A white woman, 28 years of age, died suddenly 3 years after developing bulbo-spinal poliomyelitis. The autopsy revealed extensive nodular and caseous tuberculous lesions throughout the left lung. The anterior horns of the spinal cord showed severe gliosis and loss of the majority of the motor nerve cells; a few cells displayed beginning "coffin" formation. Several *muscles* were normal except for fibrillary splitting and focal proliferation of a few sarcolemmal nuclei. Most muscles, however, exhibited advanced atrophy of all fibers, proliferation of sarcolemmal nuclei with formation of plasmodia (Fig. 16), slight to moderate patchy fibrosis, and infiltration with adipose tissue. The muscle sub-

stance varied from pale pink to dark red, and some of the narrowest fibers were cross-striated while others were homogeneous. Occasional fibers were moderately large, pale, and granular; many were beaded or fragmented. Fatty changes were not evident. Many *nerve* fibers were devoid of myelin; other fibers displayed foamy myelin. Normal fibers were numerous. In some nerve bundles the axis cylinders were greatly swollen. Endoneural collagen fibers were diffusely increased.

Recently two additional cases of bulbar poliomyelitis, with a duration of 2 and 7 days, respectively, were examined. Both showed extensive fatty metamorphosis in all muscles examined.

Discussion

The sequence of histologic changes in the muscles was as follows: The immediate alterations consisted mainly of minor variations in the staining qualities of the muscle fibers, fibrillary splitting, slight focal proliferation of sarcolemmal nuclei, and occasional focal rarefaction or necrosis of the contractile substance. Between the second day and the second week numerous fat droplets appeared within the majority of the muscle fibers, and these droplets were either oriented along the anisotropic bands or scattered diffusely. The number of fibers containing neutral fat had decreased by the second month, and such fibers were rarely present in late cases. The silver stain showed disorganization and depletion of the argentophilic granules which in normal muscles are abundant and impregnate the anisotropic bands. Atrophy of the muscle fibers of varying degrees became apparent by the second month and was pronounced in the chronic cases. In some muscles the fibers were severely atrophic in broad fields, but in most areas the degree of atrophy varied from fiber to fiber. Sometimes, normal or hypertrophic fibers were intermingled indiscriminately with others in all stages of shrinkage. The muscular devastation was so generalized that a clear-cut relation to individual motor units seldom was apparent. The amount of narrowing of the fibers in different muscles from the same patient also showed great variation. After 1 year the proliferation of sarcolemmal nuclei became prominent. Their distribution was diffuse, in clumps or plasmodia, or at the margins of fragments of muscle fibers. The changes in the staining qualities of both the atrophic and the hypertrophic fibers included paling, fine granularity, and circumscribed rarefied or vacuolated areas, which were filled with as many as twenty pyknotic distorted nuclei. In one case, these plasmodia were surrounded by granules of fat. Other fibers were either gray or hyperacidophilic. In several instances, in late as well as early cases, some muscle fibers

showed coagulation necrosis with leukocytic reaction. Cross striations were sometimes indistinct or undetectable, but on the whole they were remarkably well preserved and were seen in many of the narrowest shrunken fibers. Cross striations, which were not readily visible in paraffin sections, were observed frequently in frozen sections. Longitudinal striations were prominent, and fibrillary splitting apart of the myofibrils was widespread. Heavy scars and webs of collagen fibrils among the muscle fibers were present, predominately in the respiratory muscles of late cases. A prominent increase in connective tissue was probably often only relative because of the great shrinkage in the bulk of the muscle fibers. We were unable to determine whether fibrosis in some instances might have been caused by minute tears and zonal disintegration from too much tension and overstretching, as emphasized by Hipps.[2] Connective tissue developing by metaplasia from muscle tissue was not observed. There was also infiltration of moderate amounts of adipose tissue. Heavy lipomatosis as described by Hipps in very chronic cases was not found. Focal accumulations of leukocytes, predominately lymphocytes, were observed in the connective tissue of the diseased muscle bundles in subacute and late cases, but this finding was not noted consistently. Vascular changes were seen occasionally, and were limited to proliferation of intimal and adventitial cells in the smaller vessels.

Muscular degeneration in poliomyelitis has a steadily progressive course; the disintegration begins early and increases over a period of months and years, as was noted also by Kopits.[1] Evidence of recent degeneration is seen occasionally after 1 year or more. In the spinal cord, too, healing may be incomplete after a similar interval, as indicated by the presence of disintegrating neurons, active glial proliferation, and degeneration of fiber tracts accompanied by phagocytosis.

Unmistakable proof of regeneration of muscular tissue was absent. True budding of fibers, which is regarded as the most convincing sign, was lacking. Clusters of nuclei within the sarcolemmal sheaths have been viewed as an evidence of regeneration, but in our opinion they are indicative of myophagia. The finding of slender, structurally normal fibers in advanced cases does not justify the interpretation that they represent new fibers. They may be shrunken pre-existing fibers, because atrophy is not necessarily accompanied by loss of striation. Likewise, hypertrophic fibers probably have developed from undamaged fibers; This was believed also by Hipps,[2] who failed to find evidence of regeneration and attributed gain in muscular strength to the presence of hypertrophic fibers. Disuse atrophy may be a component of the process, but in this condition the pleomorphic character and discontinuity of

muscle fibers, which are predominant features of poliomyelitis, are not seen.

Nerve changes were easily recognized by the third day of the disease; however, they did not increase in severity in proportion to the degree of degeneration in the muscles. The principal alteration was patchy or stripe-shaped demyelination. The presence of abundant neutral fat in the myelin sheaths was pronounced in the case of 16 days' duration. Loss of tingibility of myelinated fibers in early cases, in the absence of histologically demonstrable neutral fat, was a remarkable feature. Changes in the axis cylinders were less obvious than in the myelin sheaths. They consisted of reduced staining qualities, irregular swelling and beading, and occasional disintegration. In most cases as early as the fourth day, proliferated endoneural fibers and minute hyaline scars were seen in some areas. A prominent feature was the large number of apparently normal nerves and of normal nerve fibers intermixed with severely damaged fibers.

The nerve changes resembled those seen in other demyelinating diseases. It is unlikely that they are simply an expression of secondary degeneration. In this form of degeneration the lesions are more severe and generalized, there is increased activity of Schwann cells and endoneural cells, the axis cylinders are more seriously damaged, and changes demonstrable in frozen sections treated with the myelin sheath and scharlach-R stains develop later. The nerve damage in our material usually appeared less severe than the muscle degeneration; the same condition was observed by Horányi-Hechst[4] in early cases and by Kopits[1] in his cases of many years' duration. Nevertheless, there appeared to be a high degree of correlation between the damage to the motor nerve cells and the changes in the muscles. Bodian[12] stated that the same correlation held in his material. Furthermore, he considered the neuromuscular degeneration to be secondary to the destruction of the motor nerve cells. This is in partial disagreement with our impression that the nerve lesions differed from those seen in wallerian degeneration. Unfortunately, it was not feasible to dissect out the exact nerves innervating the portions of the muscles which were obtained for study.

An interesting question regarding the degeneration of the musculature is whether the changes observed in poliomyelitis correspond to those described in the group of central atrophies or to those in primary muscular dystrophy. Wohlfahrt and Wohlfart[11] emphasized the histologic differences between the two forms. In the central types, scattered fields of atrophic fibers were found in normal muscle. There were a few

hypertrophic fibers, and these occurred singly. Marginal nuclei were increased numerically, and central nuclei were found in connection with the splitting of muscle fibers. In the peripheral types, there was an irregular intermingling of hypertrophic, atrophic, and normal fibers. Hypertrophic fibers always occurred, and they were usually very large. Hyalinization and vacuolation were found consistently; marginal nuclei did not proliferate, but central nuclei were numerous. Both types were complicated by the presence of small groups of narrow fibers that had resulted from splitting. If we apply these criteria to our material, it is difficult to place poliomyelitis exclusively in the one group or in the other. Many muscles in poliomyelitis resembled the central form of atrophy, but others, especially in cases 9 and 11, were compatible with the peripheral type.

Another aspect of the problem is whether the muscular changes in poliomyelitis are related to those which accompany peripheral nerve injuries. Bowden and Gutmann,[13] in their study of denervated human muscles, found progressive but unequal shrinkage of muscle fibers with a loosened arrangement of the myofibrils. In contrast to our cases, vascular changes were prominent, fibrosis was more extensive, and fatty degeneration was not observed. Extreme degrees of atrophy and fragmentation developed only after 3 years.

The possibility that the lesions are caused by direct action on the muscle by the virus must be considered in view of the rapidly developing severe changes in poliomyelitic muscles, the histologic dissimilarities to lesions of denervation, and the occasional resemblance to progressive muscular dystrophy, an essentially peripheral muscular disease. The recovery of virus from the muscles of experimental animals by Sanz Ibáñez[14] seems to point in the same direction. Carey and co-workers[7] clearly demonstrated dissolution of motor end-plates followed by centripetal spread of the degenerative process in the axon. In contrast, surgically severed nerves showed changes that did not begin in the end-plates but involved all parts of the nerve simultaneously. The electromyographic studies of Hodes[15] likewise emphasized the importance of deficiencies of the myoneural junctions. On the other hand, the degree of deterioration of the muscles correlated well with the severity of poliomyelitic damage of the motor nerve cells. This demonstrates the interdependence between spinal and muscular lesions. Whether the virus produces irreversible changes in the motor nerve cell before muscle changes occur is open to question. Osborne and co-workers[16] showed that repeated vigorous electrical stimulation of the paralyzed muscles of patients acutely ill of poliomyelitis prevented atrophy for many

months. This would indicate that early muscular damage, whether it is due to direct virus action or is secondary to nervous lesions, is not irreversible, provided innervation were re-established.

Summary

Voluntary muscles and peripheral nerves of 14 fatal cases of acute and chronic poliomyelitis were examined histologically.

The muscular changes consisted of early fatty metamorphosis, followed by varying degrees of degeneration, atrophy, and late fibrosis. Plasmodial formations of sarcolemmal nuclei in connection with disintegration of muscle fibers were seen frequently. Hypertrophic fibers were found in late cases. Proof of true regeneration was lacking. The histologic picture, as a rule, resembled that of the neurogenic forms of muscular atrophy, but occasionally was reminiscent of that of progressive muscular dystrophy.

The nerves showed very early stripe-shaped demyelination and relatively minor change in the axis cylinders. The lesions in the nerves appeared less severe and less extensive than those in the muscles. The pathologic process appeared to be different from wallerian degeneration.

The possibility of direct action of the virus on muscles and nerves must be considered.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 122

FIG. 1. Case 5. Biceps brachii muscle. Varicolored staining reaction. Duration of disease, 7 days. Hematoxylin and eosin stain. × 90.

FIG. 2. Case 6. Gastrocnemius muscle. Fat granules in alternate fibers. Duration of disease, 16 days. Scharlach-R stain. × 160.

FIG. 3. Case 6. Intercostal muscle. Fatty changes of muscle and nerve fibers. Scharlach-R stain. × 90.

FIG. 4. Case 9. Biceps femoris muscle. Advanced atrophy, fragmentation of fibers, and plasmodia formation. Duration of disease, 14 months. Hematoxylin and eosin stain. × 225.

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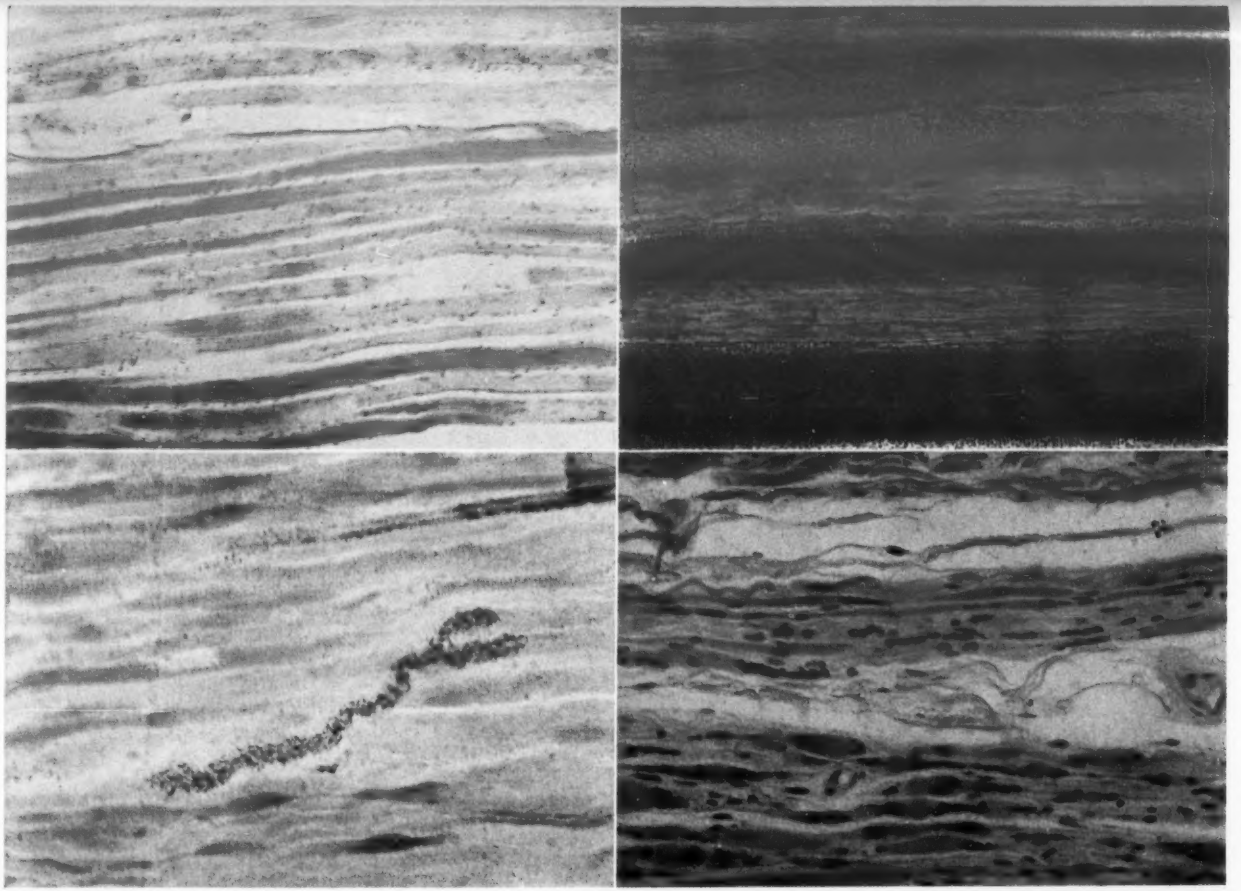
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PLATE 123

FIG. 5. Case 3. Femoral nerve. Demyelination of many fibers. Duration of disease, 4 days. Spielmeyer stain. × 90.

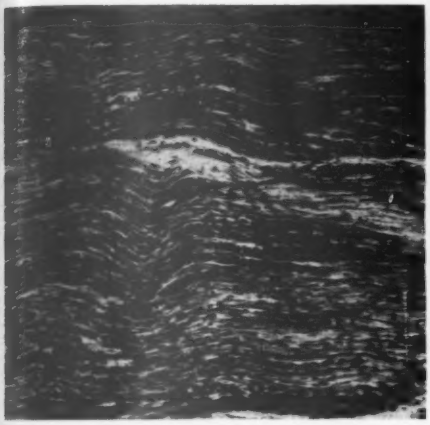
FIG. 6. Case 10. Brachial plexus. Demyelination and vacuolation of myelin. Duration of disease, 16 months. Spielmeyer stain. × 223.

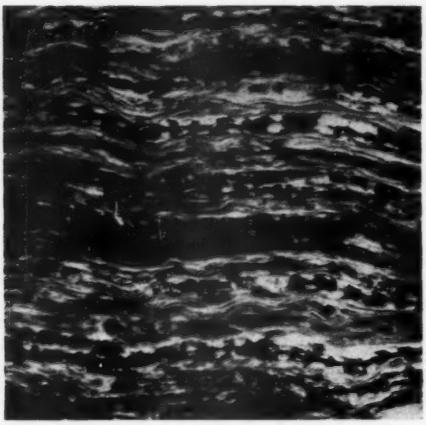
FIG. 7. Case 6. Fibrillary splitting of a muscle fiber. Duration of disease, 16 days. Hematoxylin and eosin stain. × 400.

FIG. 8. Case 6. Depletion and scattering of argentophilic granules with loss of cross striation. Braunmühl stain. × 430.

FIG. 9. Case 7. Intercostal nerve. Focal hyaline scarring. Duration of disease, 23 days. Hematoxylin and eosin stain. × 90.

FIG. 10. Case 8. Deltoid muscle. Atrophy and waviness of fibers. Duration of disease, 60 days. Van Gieson's stain. × 200.





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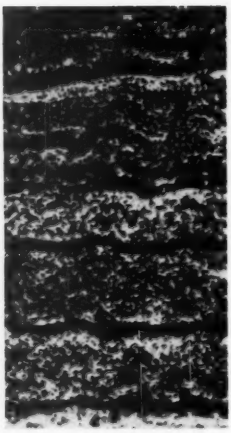

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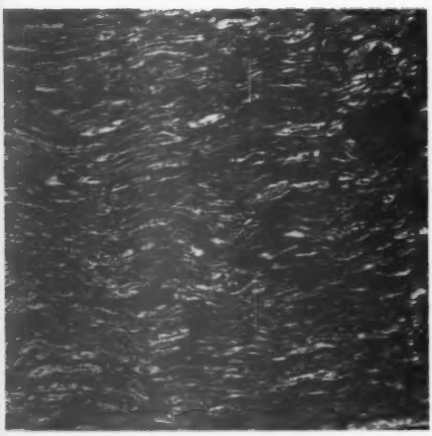

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PLATE 124

FIG. 11. Case 9. Biceps brachii muscle. Necrosis of a fiber with leukocytic reaction. Duration of disease, 14 months. Hematoxylin and eosin stain. × 400.

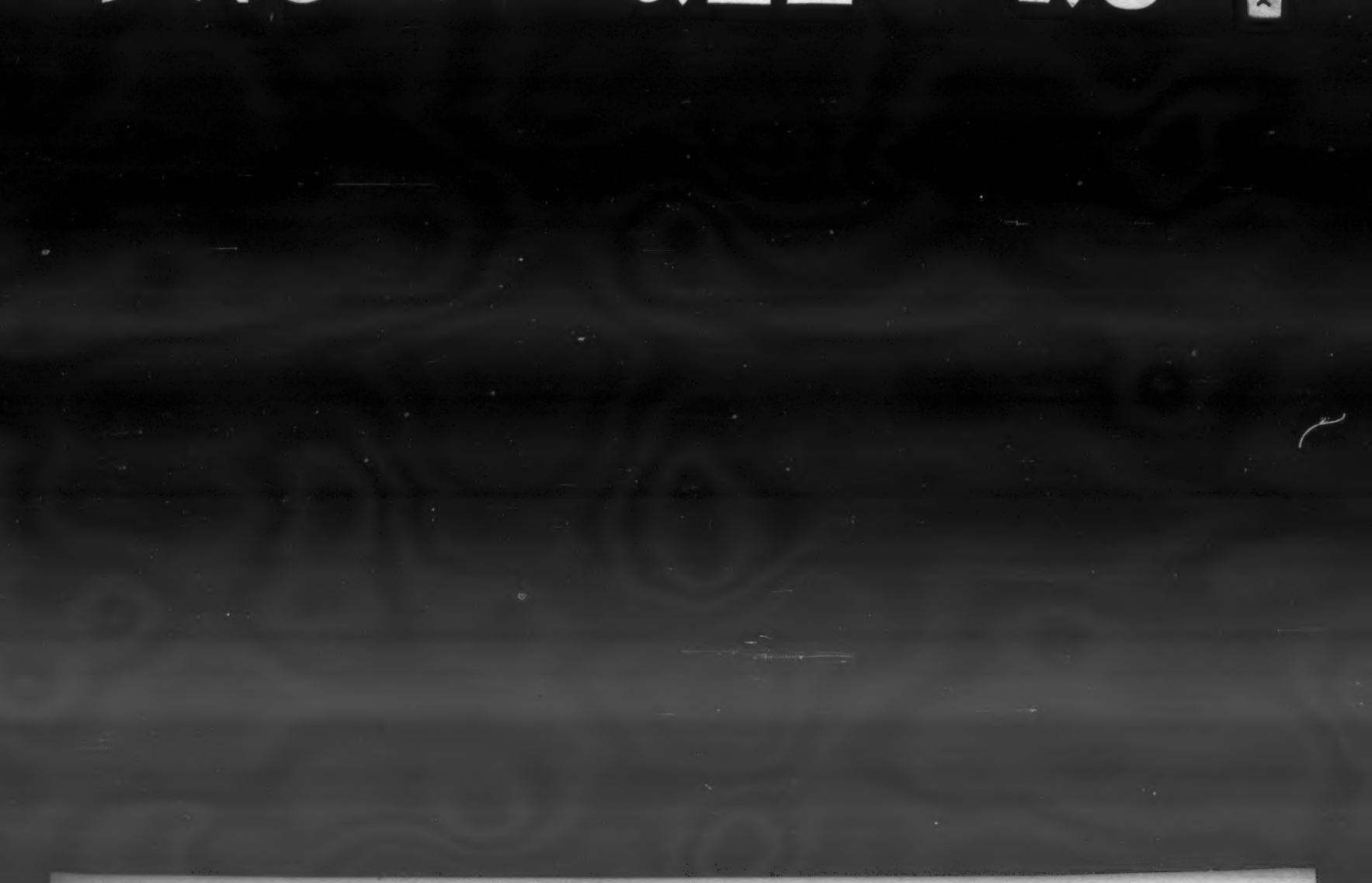
FIG. 12. Case 9. Intercostal muscle. Hypertrophic fibers showing loosened arrangement of myofibrils and vacuoles. Other fibers exhibit advanced degeneration. Hematoxylin and eosin stain. × 400.

FIG. 13. Case 11. Pectoral muscle. Intermixture of hypertrophic and atrophic fibers. Duration of disease, 32 months. Hematoxylin and eosin stain. × 90.

FIG. 14. Case 10. Psoas muscle. Fairly uniformly atrophic fibers and lymphocytic infiltration. Duration of disease, 16 months. Hematoxylin and eosin stain. × 90.

FIG. 15. Case 11. Gastrocnemius muscle. Bundles of atrophic fibers interdigitated with hypertrophic fascicles. Duration of disease, 32 months. Hematoxylin and eosin stain. × 90.

FIG. 16. Case 12. Biceps brachii muscle. Pale contractile substance, discontinuity of fibers, and proliferation of nuclei. Duration of disease, 3 years. Hematoxylin and eosin stain. × 360.



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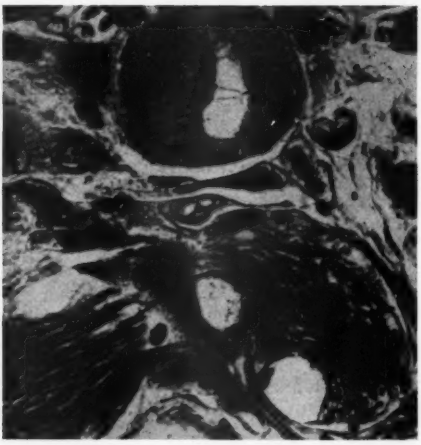

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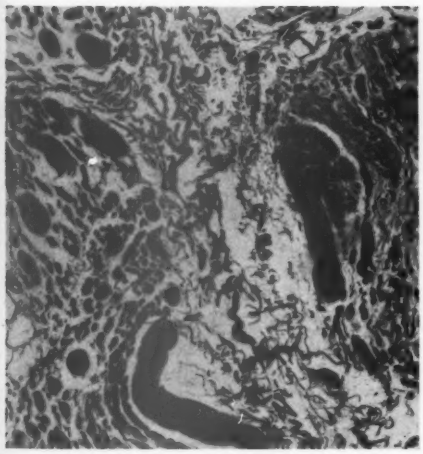

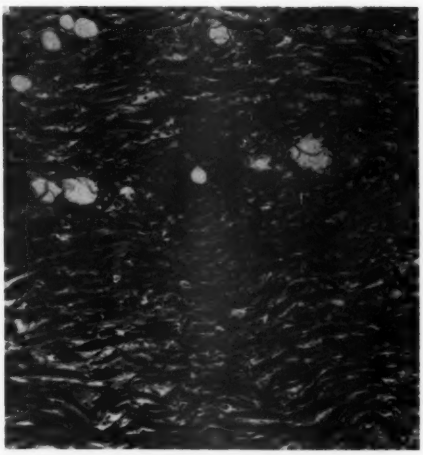

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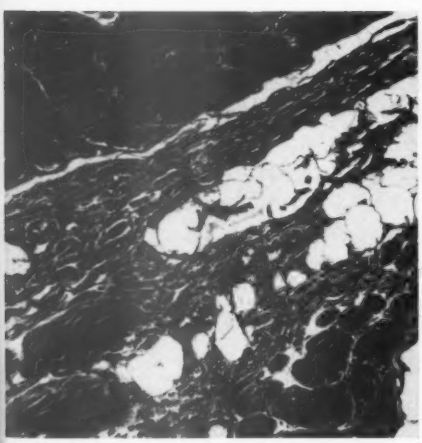

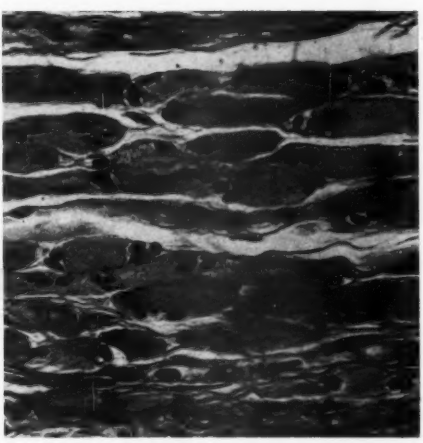

16

TRANSFUSIONAL SIDEROSIS *

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The bone marrow in certain conditions fails to produce a constant quota of adequate red blood cells. In these disorders, the wear and tear of erythrocytic ageing eventually gives rise to anemia. To date, the only recourse available in sustaining these patients is through blood transfusions. Life has been maintained over a period of months or years by frequently repeated homologous blood infusions. This is particularly true in patients suffering from diverse anemias of a refractory, hemolytic, or subleukemic nature. With numerous whole blood transfusions, a new hazard of therapeutic origin apparently has arisen to complicate these hematologic disorders. Schwartz and Blumenthal[1] referred to this condition as "exogenous hemochromatosis."

Although it is likely that a greater number of pathologic "formes frustes" exist, a few cases of severe anemia treated by repeated blood transfusions may develop overt features of hemochromatosis. In this communication, 3 previously reported[2] examples of hemosiderosis in refractory anemia are re-assessed, and 5 additional cases of transfusional iron storage disorder are reported.

Report of Cases

Case 1

W. H., a male, 66 years old, blood group O, Rh +, noted marked weight loss and fatigue 2 years before death. Six months prior to death, myelofibrosis was found by sternal trephination, with myeloid metaplasia of an axillary lymph node, pronounced anemia, and a persistent leukocytosis around 20,000 with a leuko-erythroblastic smear. Splenomegaly, hepatomegaly, and thrombocytopenic hemorrhages also were found. In the last week of life, the white blood cell count rose to 170,000 with a great increase in promyelocytes. Thirty-two transfusions, each of 500 cc. of compatible whole blood, had been given without clinical reaction.

At autopsy, there was the intense hyperplasia of myeloid leukemia in costal, sternal, and vertebral marrow. Focal osteosclerosis was found principally in the vertebrae and sternum. Leukemic infiltrations of liver, spleen, lymph nodes, and kidney were found. An olive-green reaction of the liver was obtained with the Prussian blue reaction. Iron storage was pronounced in the Kupffer cells (Fig. 1) along with minimal dusting with hemosiderin in the parenchymal cells.

Case 1, interpreted originally as myeloid metaplasia and myelosclerosis, terminated as leukemia. The principal iron storage was in the

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Kupffer cells and in reticulo-endothelial elements in other viscera. The anemia of leukemia[3] apparently results from abnormal functioning rather than ablation of the red cell-forming marrow. It may be that there is an enzyme deficiency of red as well as white cell marrow with an inability to re-utilize hemoglobin-iron and consequently a defective production of the red cell series. As to the amount of iron found in the liver in patients suffering from leukemia, it was found in reviewing leukanemia,[4] in which the anemia is of a striking character, that no mention of hemosiderosis was made. Although slight hemosiderosis of the viscera in leukemia has been noted by Jaffé[5] and Kress,[6] Whipple and Robscheit-Robbins[7] failed to find a significant increase in the iron content of the liver in acute leukemia. Collins and Rose[3] stated that siderosis was not conspicuous in their autopsy material. Direct evidence of increased hemolysis in leukemia is generally wanting.[3] It was considered, then, that in this case of myeloid leukemia treated by multiple blood transfusions, the hemosiderosis present was not associated with the natural evolution of the leukemia.

Cases 2, 3, and 4

Cases 2, 3, and 4 have been reported previously.[2] They are included in Tables I and II, and advantage has been taken of them in developing the discussion. For clinical and pathologic summaries, the earlier report may be consulted.

These cases represent examples of "blocked" marrow, due either to a non-functioning hyperplasia or to sclerosis. Life in these patients was maintained by repeated transfusions with the gradual accumulation of iron in the liver and other storage depots.

Case 5

M. R., group A, Rh +, was a male, 49 years old, who 2 years prior to hospitalization had shown pallor, weakness, and shortness of breath. Fifteen months before, severe anemia and hepatosplenomegaly were discovered. Hemoglobin was 32 per cent; red blood cells, 1,830,000; white blood cells, 2,400; and the sternal marrow revealed myelosclerosis. There was a history of industrial exposure to aniline dyes over many years. Treatment consisted of repeated blood transfusions. Leukopenia with an increase in immature red and white blood cells, anisocytosis, macrocytosis and polychromasia have been persistent. Red cell fragility was normal and Coombs' test repeatedly negative. Patient has received to date 67 (500 cc.) whole blood transfusions.

Biopsy of the liver showed pronounced siderosis (Fig. 2), intrasinusoidal megakaryocytic myeloid metaplasia, and early periportal fibrosis, providing confirmation of the clinical diagnosis and indicating steady progression of the disease. The early fibrotic changes were developing in a liver heavily laden with iron. This patient is continuing on blood transfusion therapy.

Case 6

J. P., a white male, 67 years old, had suffered from severe anemia for 2 years. A hemoglobin of 10.0 gm., maintained therapeutically; a red blood cell count of 2,500,000, and leukopenia had persisted over the same period of time. Destructive lesions of the thoracic vertebrae were discovered by roentgenologic examination. Biopsy of the sternal marrow revealed numerous myeloma cells. Urinalysis did not show Bence Jones proteins. The patient, group A, Rh +, received 27 transfusions of 500 cc. of whole blood without demonstrable reactions. Death was due to terminal bronchopneumonia.

At autopsy there was disseminated osseous myelomatosis. The liver was brown, weighed 2300 gm., and showed hemosiderosis and early periportal fibrosis with fatty metamorphosis. Focal hemosiderotic deposits were noted in the spleen. There was no hemosiderosis in kidney, pancreas, or skin.

In case 6 the severe anemia associated with plasma cell myelomatosis was treated with repeated whole blood transfusions. Hemosiderosis is not a noteworthy lesion in uncomplicated myelomatosis. The deposition of iron was principally in the Kupffer cells of the liver and in the reticulo-endothelial cells of the spleen. The early cirrhosis was of the diffuse hepatic (nutritional) type.

A similar case of multiple myelomatosis in a 60-year-old white male with severe anemia who had received 18 transfusions showed moderate siderosis of the liver on histopathologic examination.

Case 7

B. C., a female, 75 years old, blood group O, Rh +, had had definite hypochromic anemia for the last 3 years of life, associated with a constant leukopenia. Hepatomegaly and tuberculous cervical adenopathy were present. Three months before death the red cell count was 830,000, and white blood cell count was 5,700. She had pitting edema of both ankles and the plasma proteins were 4.01 gm.; albumin, 2.16 gm.; globulin, 1.85 gm. Nine blood transfusions of compatible whole blood were given during the last 3 months of life. Slight clinical reactions were elicited by the second and ninth transfusions.

At autopsy the liver was the color of bronze, weighed 1350 gm., and showed moderate granularity. Marked siderosis of liver and spleen was present. The marrow was hyperplastic and of erythroblastic type.

In case 7 we feel that a nutritional deficiency played a significant rôle in the development of pigmentary cirrhosis. This contention is well supported by the finding of hemosiderosis in the Belsen victims. Further support is offered by a case recently autopsied of an 85-year-old female who had subsisted for years on "tea and toast" and developed a long-standing severe refractory anemia. No transfusions were administered in this case. The liver showed diffuse cirrhosis and contained 22.5 gm. of iron. In addition, there was siderosis of the pancreas and periportal lymph nodes. This latter example clearly illustrates that some other

source of iron may play a prominent rôle in some cases. In Chesner's[8] case and in our case 8, there were 40 gm. of liver iron above and beyond the total amount of transfusional iron.

Case 8

T. N., a white male, 63 years old, blood group A, Rh +, had suffered from a severe anemia for the last 2 years of his life. This anemia was refractory to intensive liver, folic acid, iron, and vitamin therapy. The red blood cell count was maintained around 2,000,000 per cmm. and the white blood cell count varied between 2,000 and 6,000 per cmm. This patient received 135 transfusions of compatible blood during the last 20 months of life. Difficulty in obtaining and administering the blood transfusions was often encountered due to induction and perpetuation of iso-immunization. One month before death the patient developed bloody diarrhea which responded promptly to amebicides. These amebicidal chemicals may have acted as noxious agents on a marrow already severely damaged. Jaundice, a possible manifestation of terminal liver failure, appeared 1 week before death. On the day before death the red blood cell count was 900,000.

At autopsy there were diffuse skin petechiae. The liver was copper-colored, slightly granular, weighed 2300 gm., and gave a strongly positive Prussian blue reaction. Splenomegaly, with a moderate amount of ectopic blood formation, was noted. Microscopically, the liver (Fig. 3) showed siderotic cirrhosis. In addition there was heavy iron deposition in the spleen, pancreas, adrenals, myocardium, lymph nodes, kidneys, and bone marrow. The marrow presented a diffuse myelofibrosis with pockets of incarcerated mature granulocytes.

Case 8 presents a morphologic duplication of idiopathic hemochromatosis. The iron level in the liver is in the upper range encountered in that disease. It is our contention that this case is comparable to those reported by Zeltmacher and Bevans[9] and by Kark.[10]

The results of chemical assays and the morphologic findings in the livers in these cases are correlated in Tables I and II.

TABLE I

The Iron Content of the Liver Compared with the Transfusional Iron for Each Case

Case	Chemical Fe (liver)	Transfusional Fe
1. W. H.	4.05 gm.	8.0 gm.
2. P. E.	4.4 gm.	8.2 gm.
3. A. J.	12.5 gm.	7.6 gm.
4. M. N.	Not done	28.75 gm.
5. M. R.	Not done (biopsy)	17.0 gm.
6. J. P.	4.6 gm.	6.8 gm.
7. B. C.	2.6 gm.	2.2 gm.
8. T. N.	75.7 gm.	33.7 gm.

Average normal total liver Fe, 0.3 gm. to 0.5 gm. (Sheldon[41]).

DISCUSSION

The problem of siderosis in patients with severe anemia treated by multiple transfusions has evoked a number of contributions to the liter-

ature in the last few years. Schwartz and Blumenthal[1] reviewed 8 cases and added 5 of their own. Robb-Smith[11] commented on 5 cases of post-transfusional hemosiderosis. Finch[12] stated in 1949 that there was a distinction between the iron metabolism of post-transfusional hemosiderosis and idiopathic hemochromatosis. Ranney and Schade,[13] and Large[14] have noted hemosiderosis following repeated transfusions. Muirhead and associates[15,16] mentioned 5 cases of iron overload-disease with fatal hepatic manifestations.

TABLE II

The Presence and Grade of Fibrosis in the Liver Compared with the Location and Intensity of Pigment Giving the Prussian Blue Reaction

Case	Fibrosis (liver)	Prussian blue (liver)	
1	0	Kupffer cells	+++
		Parenchyma	+
2	0	Kupffer cells	+++
		Parenchyma	++
3	0	Kupffer cells	+
		Parenchyma	++
4	++	Kupffer cells	+
		Parenchyma	+++
5	+	Kupffer cells	+
		Parenchyma	+++
6	+	Kupffer cells	++
		Parenchyma	+
7	+	Kupffer cells	++
		Parenchyma	++
8	+++	Kupffer cells	+
		Parenchyma	+++

In all cases analyzed in this communication, the amount of iron found in the liver alone has been greater than the total amount of iron in the normal human body. The hepatic iron has reached amounts comparable to those found in idiopathic hemochromatosis. At autopsy, in each case a rusty-brown color of the liver was noted and a gross Prussian blue reaction was obtained. Five cases showed an unusually fine granularity and increased firmness of the liver. Histopathologic examination of the viscera showed the greatest concentration of hemosiderin in the liver. In the least transfused cases this deposition was heaviest in the Kupffer cells (Fig. 1); apparently the parenchymal cells are involved later. In the early stages of parenchymal hemosiderin deposition, there is a light dusting of the entire liver lobule (Fig. 2). Centrilobular necrosis and collapse are pronounced at this stage. In the later phases the pigment reaches its greatest concentration at the periphery of the liver cord and in the periportal stroma (Fig. 3). This fibrosis is a true proliferation

and not a condensation of reticulin. There were no morphologic evidences of viral hepatitis.

The pathogenesis of pigment deposition in these cases has not been completely explained. Laboratory evidence of accelerated destruction of the patients' or of donors' blood was lacking in these cases. Hemolytic transfusion reactions were conspicuously absent. At no time did transfusional jaundice develop. Bomford and Rhoads[17] stated that there are patients in the refractory anemia group in whose disorder hemolysis is particularly rapid, possibly due to hypersplenism. As far as could be elicited by indirect evidence, this factor was not acting in a decisive rôle in our cases. Although hemolysis of transfused blood may be a factor, cases 3, 7, and 8 showed more iron within the liver alone than could be accounted for by hemolysis of all the transfused blood. The absence of hemosiderosis of the kidneys, or at most only a minimal deposition, is additional evidence against an abnormal degree of hemolysis being the etiologic factor and is in keeping with a "storage" phenomenon. It is probable, then, that the siderosis is the result of a disturbance in iron metabolism rather than of increased blood destruction.

The problem in iron metabolism is to keep the accepted level of iron in the body constant either by control of absorption or by utilization. After erythrocytic destruction, it has been shown by means of labeled iron that hemoglobin synthesis depends upon recapturing freshly liberated hemoglobin-iron rather than the recall of iron from reserve stores.[18] Furthermore, it is known that in refractory anemias only 1.5 per cent of intravenous iron is utilized for hemoglobin synthesis.[19] With no excretion of iron even in patients receiving multiple transfusions,[20] there must be an intense storage of iron-rich protein,[21] particularly in the liver which is the principal storage organ of the body. The iron stored in untreated addisonian anemia is released when the hyperplastic marrow "block" is relieved by liver extract.[22] Such a mechanism is apparently not available in the diverse anemias under consideration. Thus there is a steady inexorable accumulation of iron in storage depots which is enhanced particularly when life is being maintained by blood transfusions.

Evidence in the past has suggested that iron absorption was controlled by the iron reserves of the body.[21] Recent work corroborates the fact that iron absorption is in direct relationship to the iron content within the body depots.[22] With absence of absorption of "mucosal" iron in individuals with high iron storage, it remains to explain why the assayed levels of hepatic iron in these cases are greater than the total possible contribution of transfusional iron. That this is true is supported by the analyses offered by Chesner[8] and more recently by Muirhead, *et al.*

(case 2).[16] Further evidence for the existence of iron from a source other than transfusions is presented through chemical assays of viscera other than liver.[16] Marked amounts of complemental iron have been found in lungs, spleen, kidneys, and pancreas. The provenance of this iron is debatable. While transfusions may supplement the mineral deposits,[23,24] such deposition of iron may take place in patients who have never been transfused (Roth,[25] Reich and Rumsey,[26] case 3 of Anagnostu[27]). Iron derived from mitochondrial breakdown,[28] or non-utilizable iron from the daily erythrocytic disintegration of the individuals' own cells may play an additive rôle in this iron storage disorder.

At what level the overload of iron is detrimental has not been established. Unexcreted excessive amounts of iron are deposited mainly in the sites of normal iron stores: liver, spleen, lymph nodes, and bone marrow. In time, the skin, kidneys, lungs, pancreas, and other tissues show a heavy iron content. In severe anemias, when the marrow is unable to utilize the blood iron received via transfusions, its disposal offers a problem. The accumulation of the excess is frequently associated with profound tissue alterations.

Siderosis and cirrhosis must be considered from various etiologic viewpoints. In the animal, it is possible to produce dietary hemosiderosis via the intestinal tract only by placing the animal upon a markedly restricted diet accompanied by excessive amounts of iron salts.[29-31] One cannot produce hemosiderosis merely by the addition of vast quantities of iron salts to a normal diet. Another facet of this problem concerns the intravenous introduction of iron. The intestinal mucosal barrier is by-passed with the eventual deposition of iron in the reticulo-endothelial system. The reticulo-endothelial barrier may become over-burdened and the parenchymal cells resorb the iron. Possibly, in the initial stage "transfusional" hemosiderosis may well be looked upon as an example of macromolecular thesaurosis[32] in which the plasma iron exists as colloidal ferric oxide. In this "transfusional" hemochromatosis, the loading of the reticulo-endothelial cells, particularly the Kupffer cells, with material giving the Prussian blue reaction is the earliest morphologic feature. The finding of lightly laden Kupffer cells even in the heavily transfused cases supports Cappell's[33] statement that there is a constant regeneration of these cells.

The dispersal and disposal of colloidal iron has been studied by Muir and Dunn,[34] Rous and Oliver,[35] Polson,[36,37] Menkin,[38] and others. None of these investigators, utilizing various iron compounds or homologous blood, were able to produce pigmentary cirrhosis by the intravenous route, although as early as 1912 Policard[39] had stated that excessive amounts of blood can lead to liver injury. This liver damage was ap-

parently due to mitochondrial variations showing increase in intracellular iron. In the words of Cappell, "It is noteworthy that in spite of the continued invasion of the portal tracts . . . . and despite the long continued and very marked degree of iron storage by the liver cells, nothing in the nature of cirrhosis has been observed." From the experimental aspect, some other factor besides the excessive deposition of iron is necessary for development of cirrhosis.

Support for Cappell's[33] views may be found in several related fields. Pure hemosiderosis of the kidneys, spleen, lymph nodes, pancreas, and liver have been noted in sickle cell anemia,[40] congenital hematoporphyrinuria,[41] and Cooley's anemia.[42] A heavy accumulation of iron has been found in a case of addisonian anemia repeatedly transfused.[43] Neither in these instances nor in severe dystrophic anemias associated with jaundice has concomitant cirrhosis been described. In the field of comparative pathology several syndromes associated with intensive siderosis of the liver, spleen, kidneys, and pancreas have been noted. Neither in "falling disease,"[44] a copper deficiency syndrome of adult cattle, nor in "enzootic marasmus,"[45] a cobalt deficiency in sheep and cattle, does cirrhosis accompany the intensive hepatic siderosis.

There are several reasons, then, for the belief that iron *per se* is not the causative agent in the production of liver fibrosis. As Gillman, Mandelstam, and Gillman[46] stated, "It is possible to have hepato-cytosiderosis with or without cirrhosis" and thus unmasked iron is an expression of deranged metabolism, which to a degree, may be reversible. By analogy, one would reason also that endogenic or exogenic iron exists in a more or less inert form in the body. Iron demonstrable as Prussian blue is not responsible for fibrosis, as noted in idiopathic pulmonary hemosiderosis (Ceelen-Gellerstedt syndrome).[47] Investigators of this disorder regard the parenchymal fibrosis as unrelated to the adjuvant iron. The inert nature of iron is also illustrated in the "benign pneumoconiosis" of arc welders[48] and in the hematite lung.[49] Finally, Gillman *et al.* have demonstrated that the pigment in human cytosiderosis is relatively inert. These workers showed by intravenous injections into cats of the actual pigment extracted from South African pellagrin livers that siderosis developed, but not cirrhosis.

Himsworth[50] has suggested that a circulatory disturbance, possibly due to stuffing of the Kupffer cells, may result in centilobular necrosis. As a result of this impediment, the slowly circulating blood is largely depleted of its nutriments by the time it has progressed some distance down the sinusoids. As a sequel, the more central cells are malnourished and ultimately degenerate and disappear. Thus it is also evident that

in some way, as yet unexplained, the products of cellular degeneration can pass within the hepatic lobule to the portal tracts. It may be that the products of the degenerating liver cells, rather than the migration of the iron pigment, are responsible for the portal fibrosis.

Observations and investigations on the rôle of nutrition in siderosis have been carried out by the Gillmans[28] and Gore.[51] The Gillmans attributed hemosiderotic liver fibrosis in pellagrins to a disturbed intracellular mitochondrial metabolism due to a dietary fault. In the experimental animal, pigmentary cirrhosis has been produced only in the presence of linked hepatic-pancreatic lesions,[52] or a poorly balanced diet.[53]

The nature and residence of the fundamental fault in this disorder are still obscure, although Schwartz and Blumenthal[1] have attributed "exogenic hemochromatosis" to a basic defect in intestinal absorption. On the other hand, it has been suggested that the excessive visceral storage may be due directly to greatly increased amounts of transfused iron introduced into the body with fibrosis as a sequel. Another explanation offered for this disorder is that since life is being maintained by donor blood, the individual lives long enough for certain cellular decadent alterations to reveal themselves in organs with a high metabolic activity through the "marker" substance iron. Sheldon[41] had previously concluded that a metabolic disturbance in the cell was responsible for the deposition of iron. Schwartz and Blumenthal admitted, "it is not inconceivable that the anoxia due to the anemia plays a contributing rôle by disturbing the intracellular metabolism, and thereby making the cells more susceptible to the deposition of iron."

Rather than the deposition of iron being directly initiative of cirrhosis, it is suggested that cirrhosis develops independently and owes its genesis to a local nutriment deficit. Gillman *et al.*, in their work on hepatocytosiderosis,[46] have stated "that in haemolytic anaemias the iron pigment in the liver cells may not be attributed entirely to the passage of altered hemoglobin or excessive iron into the liver cell. The anaemia itself can alter the nutrition of the tissue." The intensity of the pigmentary cirrhotic process may depend upon the duration and severity of the anemia and local nutritional deprivation and not solely upon excessive therapeutic blood transfusion.

Summary

Eight patients with severe anemia of diverse causes were treated by multiple blood transfusions. Post-mortem observations revealed histologic and chemical evidence of pronounced iron storage. Direct hemol-

ysis was not responsible for this iron overload. Five of these cases showed granular livers; 2 gave chemical assays of iron comparable to the levels encountered in conventional hemochromatosis.

Case 3, 7, and 8 assayed more iron in the liver alone than the total contribution of transfused iron. The source of this extra-transfusional iron is debatable but the amount of iron present in some of these examples suggests some continuance of intestinal absorption and storage. Since these anemic patients are "kept alive" by repeated blood transfusions, it is quite possible that cellular degenerative processes and local nutrient deficits are partially responsible for tissue siderosis and fibrosis.

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DESCRIPTION OF PLATES

PLATE 125

FIG. 1. Case 1. Intense accumulation of iron pigment in the Kupffer cells and minimal dusting of the liver cord cells. Hematoxylin and eosin stain. × 230.

FIG. 2. Case 5. Diffuse heavy deposition of iron pigment in the parenchymal cells and stroma. Prussian blue stain. × 470.

Xi

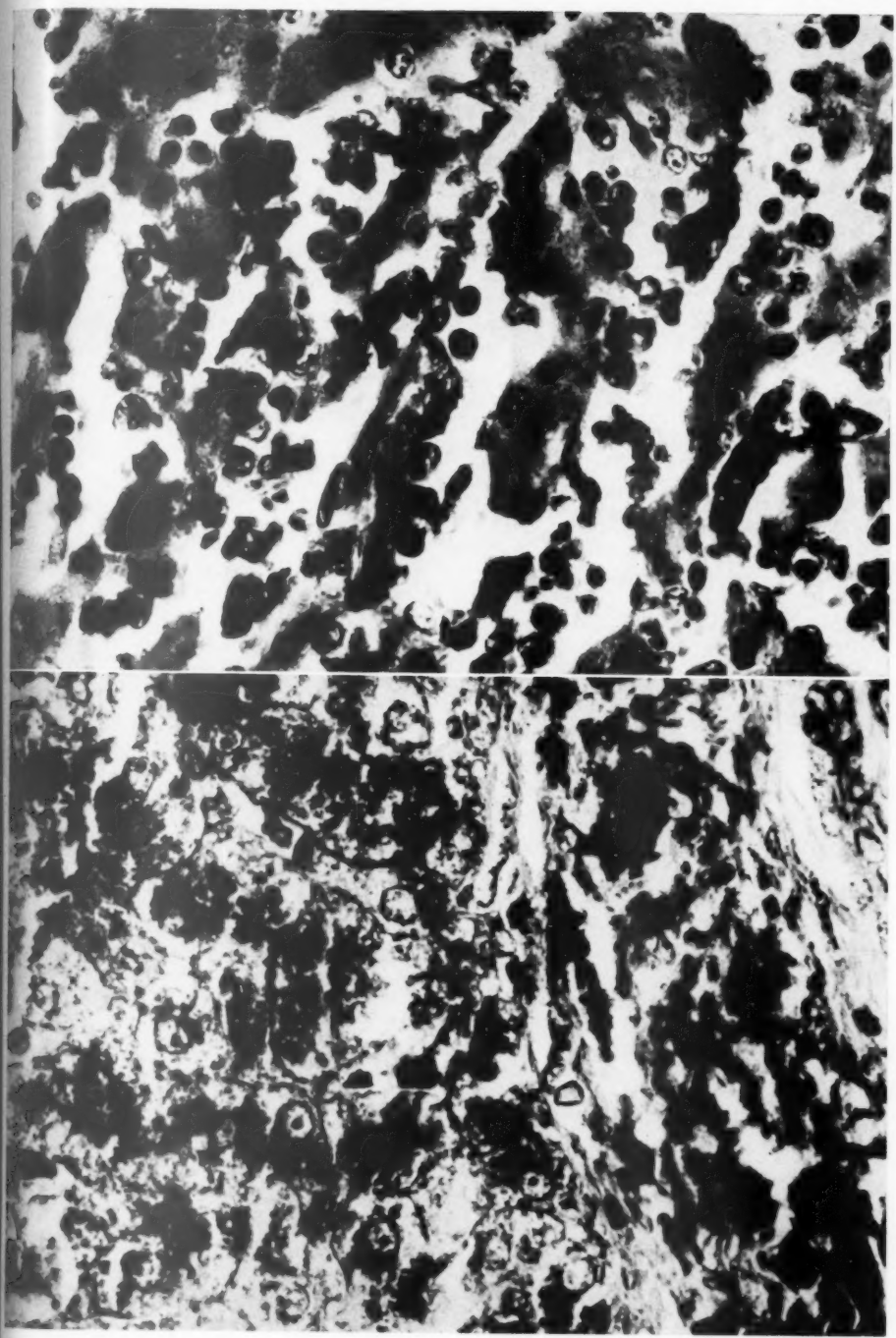
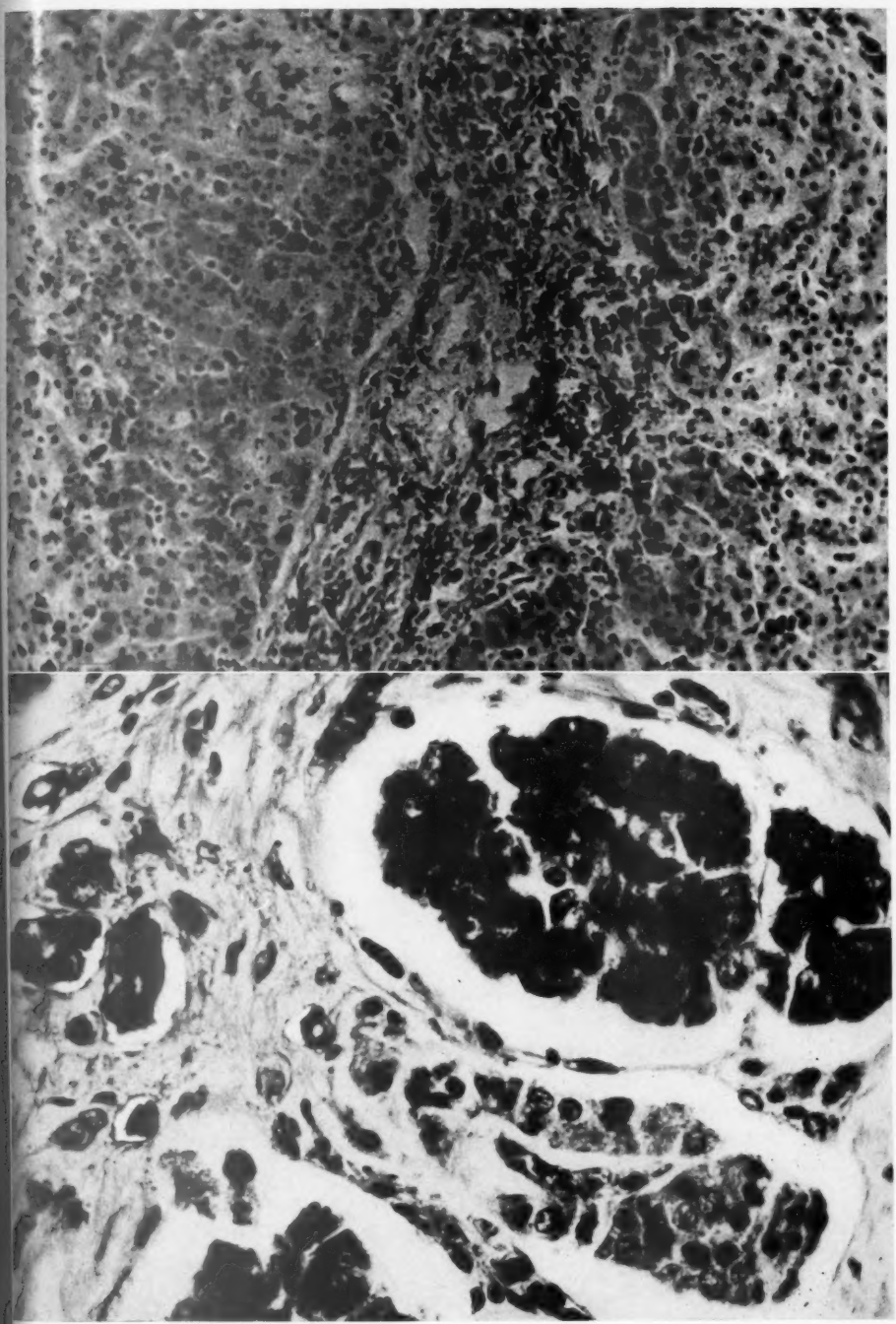


PLATE 126

FIG. 3. Case 8. Siderosis and cirrhosis are both present. Pigment is concentrated particularly at the periphery of the liver lobules and in the periportal stroma. Hematoxylin and eosin stain. × 120.

FIG. 4. Case 8. Several pancreatic acini heavily laden with hemosiderin pigment. Hematoxylin and eosin stain. × 400.

XVIII



Wyatt, Mighton, and Moragues Transfusional Siderosis

RENAL SIDEROSIS IN HEMOGLOBINURIC NEPHROPATHY *

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The deposition of iron pigment in the kidneys of patients suffering from diseases associated with intravascular hemolysis is widely known. Thus, hemosiderin may be found in the renal tubular epithelium in cases of blackwater fever,[1] Marchiafava-Micheli syndrome,[2] transfusion reaction due to incompatible blood,[3] hemolytic anemia,[4] post-abortal oliguria,[5,6] and sulfonamide hypersensitivity reaction.[7] In these conditions lysis of erythrocytes is accelerated and transcends the physiologic mechanisms which are normally capable of utilizing or excreting hemoglobin and its derivatives. It is under these circumstances that the pathologist finds the morphologic evidences of spilling over and of accumulation of hemoglobinogenous pigments in the body tissues.

With this in mind, a study was undertaken to determine the incidence in patients with hemolysis of renal hemosiderosis which was sufficiently severe to produce renal impairment, and to evaluate the constancy of such pigmentation as a morphologic criterion in differentiating these conditions from renal insufficiency due to crushing injuries. Such a distinction would tend to accentuate the already recognized etiologic and pathogenic differences between the two main subdivisions of lower nephron nephrosis, namely, hemoglobinuric nephrosis and the crush syndrome.

MATERIAL

From the autopsy material at the University Hospital (Michigan), 9 examples of renal insufficiency secondary to intravascular hemolysis were selected. Each clinical record revealed that the patient had developed acute renal failure with oliguria or anuria subsequent to an adequate precipitating factor. The non-protein nitrogen values had ranged from 70 to 235 mg. per 100 cc. of blood and the urine had given a positive benzidine test, in the cases in which these tests had been made. The patients had survived from 36 hours to 10 days after the onset of symptoms. Post-mortem examination of the kidneys had revealed renal findings compatible with those described for the lower nephron syndrome: degeneration and necrosis of the epithelium of the distal convoluted tubules and ascending limbs of Henle, formation of heme casts in the distal and collecting tubules and precipitation of an eosinophilic amorphous material in the glomerular spaces and proximal convoluted tubules. In addition, the kidneys of the patients who survived for the

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longer periods had developed interstitial edema and inflammatory cellular exudate.

Microchemical tests for iron were done on sections of kidney in order to determine the presence of renal hemosiderosis, and on sections of liver and spleen in the hope of obtaining more information concerning the nature of the underlying hemolytic process. The autopsy material was fixed in 10 per cent formalin, and Gomori's iron reaction was the method employed.

The pH of the fixative approached neutrality, ranging from 5 to 8. This is significant because a pH range of 3.5 to 4 may induce post-mortem intravascular laking of erythrocytes with the formation of hemoglobin degradation products.[8] In all cases the autopsies were performed soon after death and selected blocks were fixed immediately, since post-mortem formation of hemosiderin has been reported as a complication of delayed fixation.[9] It was noted in this respect that erythrocytes seen in the vessel lumina were intact and in no instance was there hemosiderin or any other hemoglobinogenous product present as an artifact, except for the frequently and easily recognized formalin precipitate of hemoglobin. Additional control was established by applying the stain for demonstrable iron to 100 kidneys from non-hemolytic cases in which red blood cells were present in the tubules. The iron reactions were negative and the erythrocytes were preserved, indicating that these cells do not become sources of hemosiderin when there has been immediate and proper fixation. For this specific approach detailed observations based on hematoxylin and eosin preparations have been omitted and emphasis is placed upon the presence or absence, degree of intensity, and anatomical distribution of iron-reacting pigment in the 9 cases.

Results

The pertinent information concerning hemosiderin deposition in the 9 cases given detailed study is presented in Table I. It is to be noted that in every case save one (case 5), the reticulo-endothelial cells of the spleen contained hemosiderin. Moreover, while the degree of splenic hemosiderosis was variable, in most instances the amount of iron was minimal.

Another variable was the anatomical site of deposition of iron-containing pigment in the liver. In 5 patients demonstrable iron was present only in the Kupffer cells; in 2 the hemosiderin was limited to the hepatic cord cells; and in the remaining cases the iron-reacting pigment was present in both parenchymal and reticulo-endothelial components. When the liver cords contained hemosiderin, it was seen to best advantage in the cells at the periphery of the hepatic lobule.

Distribution of Hemosiderin in Kidney, Liver, and Spleen as Related to Clinical Data in the Nine Cases Studied

Case	Diagnosis	Renal function	Transfusions	Survival	Additional clinical notes	Kidney	Liver	Spleen
1 W. L. A-367-AZ	Acquired hemolytic anemia (crisis)	Anuria; nonprotein nitrogen, 122 mg./100 cc.	Two transfusions after onset of anuria (no reaction)	8 da.	Surgical absence of spleen for 3 years	Diffuse blue-reacting and granular hemosiderin in lumina and in tubular epithelium	Granular hemosiderin in Kupffer cells	(Surgical specimen) moderate hemosiderin deposition in reticulo-endothelial cells
2 K. L. A-61-AT	Hemolytic anemia (crisis)	Hematuria and oliguria; nonprotein nitrogen, 70 mg./100 cc.	One transfusion 48 hrs. before death (no reaction)	72 hrs.	Hemoglobin, 29%	Diffuse blue-reacting and granular hemosiderin in lumina and in tubular epithelium	Granular hemosiderin in liver and Kupffer cells	Mild deposition of hemosiderin in reticulo-endothelial cells
3 J. N. A-354-AN	Meningococcic meningitis	Anuria; blood urea nitrogen, 158 mg./100 cc.	One transfusion, 320 cc. (severe reaction)	7 da.	Severe hemolysis	Diffuse blue hemosiderin in lumina of tubules	Slight deposition of hemosiderin in Kupffer cells	Moderate deposition of hemosiderin in reticulo-endothelial cells
4 F. S. A-10-AY	Comminuted supracondylar fracture of left humerus	Severe oliguria; nonprotein nitrogen, 151 mg./100 cc.	One transfusion, 350 cc. (severe reaction)	8 da.	Severe hemolysis	Diffuse blue hemosiderin reaction in lumina and in epithelium of tubules	Granular hemosiderin in liver cells	Mild deposition of hemosiderin in reticulo-endothelial cells
5 E. H. A-428-AO	Pernicious anemia in relapse	Anuria	One transfusion (severe reaction)	36 hrs.	Severe hemolysis	Diffuse blue and granular hemosiderin in tubular epithelium	Granular hemosiderin in liver cord cells	Absent
6 G. J. A-114-AT	Subacute bacterial endocarditis treated with sulfadiazine	Hematuria and oliguria; nonprotein nitrogen, 81.8 mg./100 cc.	None	8 da.	Severe hemolytic hypersensitivity reaction to sulfadiazine	Diffuse blue reacting hemosiderin in lumina	Granular hemosiderin in liver and Kupffer cells	Mild deposition of hemosiderin in reticulo-endothelial cells
7 L. D. A-267-AY	Otitis media treated with sulfadiazine	Anuria	One transfusion 1 day before death (no reaction)	7 da.	Hemolytic hypersensitivity reaction to sulfadiazine	Diffuse blue and granular hemosiderin in lumina and tubular epithelium	Hematin-like material in Kupffer cells	Mild deposition of hemosiderin in reticulo-endothelial cells
8 E. B. A-13-BA	Abortion	Blood urea nitrogen, 235 mg./100 cc.	None	6 da.	Severe hemolysis	Diffuse blue hemosiderin reaction in lumina of tubules	Mild deposition of hemosiderin in Kupffer cells	Mild deposition of hemosiderin in reticulo-endothelial cells
9 G. D. A-289-LBA	Abortion	Severe oliguria	None	10 da.	Severe hemolysis	Diffuse blue hemosiderin in lumina of tubules	Marked hemosiderosis of Kupffer cells	Mild deposition of hemosiderin in reticulo-endothelial cells

Renal deposition of hemosiderin was present without exception in each case of the study group. However, in the microscopic appearance there were both qualitative and quantitative differences in individual kidneys. The iron-reacting pigment appeared in two distinct forms, one giving a uniform, diffuse, pale blue, non-granular staining reaction and the other presenting as a finely to coarsely granular dark blue material. The intensity of pigmentation varied in individual patients. The hemosiderin was found either in the lumina or in the epithelium of the proximal convoluted tubules, or in several instances in both lumina and epithelial cells. Other levels of the nephron did not participate in the siderosis. In the lumina of the proximal convoluted tubules the stain made evident a uniformly pale blue, amorphous substance, giving the impression that the iron-containing pigment might be partially masked by a protein substance. The uniform, diffuse, blue coloration was seen also in the proximal convoluted tubular epithelium, while in some cells blue granules of hemosiderin also were present. The diffuse reaction usually involved all the epithelium of a given proximal tubule, whereas the granular material was focal in distribution. In several kidneys granular hemosiderin was found in the lumina of the tubules. Individual cases varied as to the number of convoluted tubules involved and in the intensity of the staining reaction.

In correlating these observations with those based on other stains, it was noted that the dark blue granules were visible in preparations stained with hematoxylin and eosin as a golden brown, finely granular pigment, while the diffuse blue coloration had no counterpart in sections subjected to routine staining. Certain investigators have stated that the latter form of pigment produces a brown color after hematoxylin and eosin staining.[8] This I could not substantiate.

Literature

There are many references in the literature to the localization of hemosiderin in the kidneys in various diseases. Dudgeon,[1] in presenting the renal lesions of the acute stage of blackwater fever, mentioned granular material in the lumina of the convoluted tubules which contained traces of free iron, or iron in large quantities. This was interpreted as a breakdown product of hemoglobin secondary to intravascular hemolysis.

Burwell, Kinney, and Finch[5] reported clinical recovery from the renal impairment which followed abortion. However, the patient died approximately 3 months later from homologous serum hepatitis. Examination of the kidneys showed brown pigment granules, which gave a positive reaction for iron, in the cells of the renal tubules and through-

out the stroma. This was thought to be an example of hemoglobinuric nephrosis due to severe hemolysis.

Payne[10] reported the death of a patient with acute hemolytic anemia who had developed renal insufficiency following a transfusion reaction. Post-mortem study revealed that the renal tubular system was blocked by brown, homogeneous coagula with aggregates of granular, brown, iron-containing pigment lying free or engulfed in macrophages. Recently, Rather[4] presented a case of acquired hemolytic anemia of unknown cause in which the necropsy findings were those of lower nephron nephrosis, disseminated fibrinoid arteritis, and atypical pneumonia. Microscopic study of the kidneys revealed numerous small brown granules which reacted to form Prussian blue in the cells of the proximal convoluted tubules. Sussman and Kayden[11] studied a patient who developed renal insufficiency due to paroxysmal hemoglobinuria from exposures to cold, and the kidney changes were those of lower nephron nephrosis. Again an iron reaction (Prussian blue) showed loose masses of material which contained hemosiderin in the lumina of the primary convoluted tubules, and in the distal nephron stronger reactions were obtained from other pigmented masses.

In another instance of renal impairment associated with severe hemolysis, Ravid and Chesner[7] described a fatal toxic reaction complicating sulfapyridine therapy. The renal lesion was compatible with lower nephron nephrosis but, in addition, the use of the Prussian blue method revealed the iron-containing pigment in the tubular casts and coarse hemosiderin granules within the cytoplasm of the tubular epithelium.

DeGowin, Warner, and Randall[3] analyzed 8 fatal cases of renal dysfunction following transfusion reactions and one case in which the patient died of hemolysis attributed to quinine. Two of this group had a considerable amount of hemosiderin in the tubular epithelium. An experimental study by the same authors[3] was concerned with the production of hemolysis by the injection of canine hemoglobin into dogs and examination of the tissues for the deposition of hemoglobinogenous pigment. They found the distribution, as determined by potassium ferrocyanide stains, to be in the Kupffer cells of the liver, the reticuloendothelial cells of the spleen, and the cells of the proximal convoluted tubules of the kidneys. In the animals which succumbed the renal changes were those now described for hemoglobinuric nephrosis. The authors concluded that the presence of pigmented casts and of hemosiderin is an important criterion in establishing the anatomical diagnosis of transfusion nephropathy. Further experimental work in intravascular hemolysis was carried out by Muir and Dunn[12] who produced hemolysis in rabbits by the intravenous injection of antiserum obtained from

a goat which had received injections of rabbit corpuscles. The study showed that the hemosiderin in the kidney produced a diffuse Prussian blue reaction with but few granules in the epithelium. In the liver there was at first a diffuse blue staining of the cord cells but later the hemosiderin became granular. The Kupffer cells also contained hemosiderin. Iron pigmentation of the spleen was limited to the reticulo-endothelial cells. I have obtained similar results with rabbits by the intravenous injection of distilled water in quantities of 200 to 250 cc., with the exception that in the liver the pigment was limited to the Kupffer cells.

Rous and Oliver[13] gave rabbits for a month daily injections of 10 cc. of citrated whole blood from other rabbits. The spleen was the first organ to show hemosiderin and served as a buffer for other tissues. Animals sacrificed at later intervals contained hemosiderin in both hepatic cord cells and Kupffer cells, and the pigment was located in the tubular epithelium of the kidneys.

On the other hand, in lower nephron nephrosis as a sequel to crushing injuries there is no demonstrable iron-reacting pigment in the kidneys. Bywaters and Dible,[14] in discussing the crush syndrome, stated that the proximal convoluted tubules were filled with amorphous material which might have been cellular débris or precipitated protein and which had no iron pigmentation. The same authors offered the opinion that the casts in the distal convoluted tubules were myohemoglobin or one of its derivatives. These findings are in accord with the observations of Newman and Whipple[15] who noted that muscle hemoglobin had a very low renal threshold and that the epithelium of the convoluted tubules did not take up a pigment staining for iron, muscle hemoglobin differing in this respect from blood hemoglobin.

In his monograph on the lower nephron syndrome, Lucké[16] was in agreement with the preceding concepts for he thought that the pigmented casts, after the destruction of muscle, were composed predominantly of myohemoglobin, whereas in hemolytic conditions, the casts were derived from hemoglobin and its degradation products. The casts seen in the distal portion of the nephrons were iron free, an observation which is in accord with my cases. But Lucké did not mention a positive iron reaction in the proximal convoluted tubules in the cases due to hemolytic conditions, a finding which is present in my series.

Mallory[17] wrote of the renal findings in 60 fatal cases of battle injury, stating that none of the material gave a positive iron reaction and concluded that the granular precipitate in the proximal convoluted tubules was probably albuminous in character. Utilizing a chemical test which distinguished myohemoglobin from hemoglobin, he found that myohemoglobin was dominant in 14 of 51 wounded patients whose

urine gave a positive benzidine test. By inference, one is led to believe that hemoglobin was found in every case and that both pigments are present after crushing injuries. If hemoglobinuria is a feature of the crush syndrome, the hemoglobin is excreted as such, since Mallory did not demonstrate intravascular catabolism of hemoglobin with degradation products passing through the glomeruli, allowing iron-reacting pigments to be absorbed by the tubular epithelium.

The material from which the study cases were selected affords but limited experience with lower nephron nephrosis on the basis of crushing injuries. However, in a recent necropsy upon a patient whose death was due to trauma with renal impairment, there were heme casts in the kidneys and tubular necrosis. These kidneys failed to reveal any iron pigmentation by special staining.

Further distinction between cases of renal insufficiency subsequent to intravascular hemolytic activity and those complicating the crush syndrome was found in the gross features of the kidneys. In our 9 fatal cases with intravascular hemolysis the kidneys were usually moist and of reduced consistency. They were reddish to chocolate brown and showed petechial hemorrhages on the cortical surface. Corticomedullary distinction was usually impaired and in no instance was there cortical swelling or pallor. The average combined weight of the kidneys was approximately 400 gm.

This latter observation is in general agreement with the results of Lucké[16] who found the combined weight of the kidneys to average approximately 485 gm. in 49 cases which followed trauma. However, he noted that the increase in size of the kidney tended to be less following hemolytic conditions. Also in his material cortical pallor was a prominent feature.

Discussion

It has been postulated by Fairley[18] that there are three methods of hemolysis. The first is thought to be an intracellular process with active phagocytosis of erythrocytes in cells of the reticulo-endothelial system —more specifically, the phagocytic pulp cells of the spleen and the Kupffer cells of the liver. Two end products result from this physiologic activity, namely, bilirubin and hemosiderin. Under certain conditions of increased red cell destruction, such as are found in many of the hemolytic anemias or following multiple transfusions, this normal route of elimination of senile erythrocytes is called upon to phagocytize more, and perhaps imperfect, red cells. The quantitative increase in end products is made evident by increase in serum bilirubin and by hemosiderosis of certain organs, mainly the liver, spleen, and lymph nodes. Essentially then, this form of hemolysis is an exaggeration of the body's

established processes. In histologic study the resulting iron pigment is confined largely to the reticulo-endothelial cells and in all probability there is no free circulating hemoglobin.

Considering the occurrence and fate of extra-corpuscular circulating hemoglobin, Fairley[18] presented a second concept of hemolysis which was primarily that of intravascular destruction of erythrocytes with release of hemoglobin and its derivatives into the blood stream. This phenomenon, not mediated through phagocytic activity on the part of the reticulo-endothelial system, involves a direct lysis of erythrocytes by toxic agents or hemolysins. It is a more severe and rapid process with a mounting level of circulating hemoglobin in the plasma until renal threshold values are reached and hemoglobinuria may result. Furthermore, the author noted a previously unidentified intermediary pigment in this form of hemolysis in cases of blackwater fever and incompatible blood transfusions, which had a distinct spectroscopic band. After identifying this new pigment, which he called methemalbumin, Fairley concluded as the result of extensive experiments that it was probably a combination of hematin and serum albumin. To date this product has been found only in the serum. This form of hemolysis is entirely pathologic and has no physiologic counterpart. It is thought to be the predominating type in our fatal cases of hemoglobinuric nephrosis, although the distribution of iron pigment tends to incriminate, to a lesser degree, the intracellular destruction of red blood cells. My observations lead me to believe that the products of the breakdown of hemoglobin occur in the circulation and produce hemosiderin or pigmentary precursors of hemosiderin, possibly in combination with serum proteins. Furthermore, these products may be deposited as material giving a pale blue reaction for iron in parenchymal structures such as the liver cord cells. In many cases of severe intravascular hemolysis, this pigment, together with intact hemoglobin, may transcend the glomerular barrier to gain entrance into the lumina of the proximal convoluted tubules. Later, the iron pigment is absorbed by the epithelium of the tubules as a part of the bodily economy in the conservation of iron. This absorption appears to be very effective, for iron is not seen in lower segments of the nephron nor do the heme casts of the distal nephron contain stainable hemosiderin.

Fairley[18] considered a third method of disposal of hemoglobin to be its excretion through the kidneys as such without intravascular catabolism. This procedure may be dependent upon low individual renal threshold levels or on the severity of the hemolytic process. In this connection I have noted the absence of iron pigment in the hepatic cord cells when it had passed through the glomeruli in 5 of the 9 cases of

hemoglobinuric nephrosis. In addition to a lower threshold value, the possibility of a reduction in intravascular catabolism of hemoglobin in these patients was recognized.

Recently, Finch, Thomas, Walsh, and Fluharty[19] presented additional evidence to support the concept of two forms of hemolytic activity. They used erythrocytes labeled with radio-iron and hemoglobin prepared from such cells to localize pigment cleared from the blood. Their results in connection with intravascular hemolysis showed that hemoglobin, regardless of its plasma level, accumulated in the kidney, and, since free hemoglobin is processed chiefly by renal tissue, supported the hypothesis that hemoglobin is normally filtered through the glomeruli and is re-absorbed by the tubules. In contrast, in intracellular destruction of erythrocytes the reticulo-endothelial system takes up the damaged red blood cells but does not handle free hemoglobin. The possibility that hemoglobin may be converted to hemosiderin within the tubular epithelial cells cannot be excluded.

Comment

Some patients with spherocytic anemia, pernicious anemia, and aplastic anemia (with multiple transfusions) in whom the cause of death is other than renal insufficiency, show hemosiderosis of kidneys, liver, and spleen. This suggests that in these diseases there are hemolytic components of varying degrees of intensity, although some workers[20] would ascribe the hemosiderosis to failure of utilization with secondary storage of the iron. It must be admitted also that visceral siderosis shows, in some of the conditions mentioned, a distribution similar to that in patients dying with renal insufficiency. However, this is not too extraordinary since we are dealing with intravascular hemolysis of different degrees of intensity; and in most cases of hemolytic activity, the rapidity and extent of erythrocytic destruction is not of such severity as to initiate lower nephron nephrosis even though some degree of hemoglobinuria may occur.

The initiation of the change which brings about renal insufficiency is dependent upon the presence of circulating hemoglobin, for this compound has been shown to exert a specific vasoconstrictive effect on the renal vessels.[21] This fact is in accord with the more recent developments in the pathogenesis of the lower nephron syndrome. Hemosiderinemia as a result of intravascular catabolism probably has a minor rôle, if any, in the development of this form of renal dysfunction. Nevertheless, it is thought that renal siderosis, a factor possessed in common by all my cases, is a stigma of the terminal episode of fatal intravascular hemolysis. In case 5, the patient, who had pernicious anemia in relapse, did not

have iron pigment in the spleen. This is occasionally true of the distribution of hemosiderin in this disease and my results raise the question whether the fatal transfusion reaction was responsible for the siderosis of the liver and kidneys. One possible explanation for this apparent deviation would be that the hemolytic process was almost purely intravascular, with minimal intracellular (reticulo-endothelial) liberation of hemoglobin. This patient, it will be noted, survived for only 36 hours.

A dual method of destruction of red blood cells explains, in part, why hemosiderin deposition was so variable in the liver. When iron pigmentation occurred in both Kupffer and liver cells it is probable that both mechanisms participated in the hemolytic episodes. Why hemosiderosis should be present in the hepatic cord cells in certain instances of intravascular hemolysis and absent in others is a problem that needs further study. It was observed also in case 7 that there was a hematin-like pigment in the Kupffer cells, which must be considered as another product of hemoglobin catabolism.

An additional theoretic consideration involves the pathogenesis of renal failure following abortion.[6] It appears that here retained placental tissue is a factor, and one can only speculate as to the possibility of syncytial hemolysins producing the hemolytic activity.

Regardless of the specific mechanisms, the iron pigmentation seen in the kidneys is of diagnostic value in assigning cases of lower nephron syndrome to the division of hemoglobinuric nephrosis.

Summary and Conclusions

Study of 9 fatal cases of hemoglobinuric nephrosis demonstrated that hemosiderin in the kidneys is of constant occurrence and serves as an anatomical criterion in differentiating this form of lower nephron nephrosis from that subsequent to crushing injuries.

These patients have in common a severe form of intravascular hemolysis in which hemoglobin and its degradation products are eliminated, for the greater part, through the kidneys. Apparently the concentration of free circulating hemoglobin is sufficient to initiate renal insufficiency.

The deposition of hemosiderin in the reticulo-endothelial tissue is a manifestation of intracellular hemolysis and enacts no significant rôle in the pathogenesis of the renal lesion.

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[*Illustrations follow*]

DESCRIPTION OF PLATE

PLATE 127

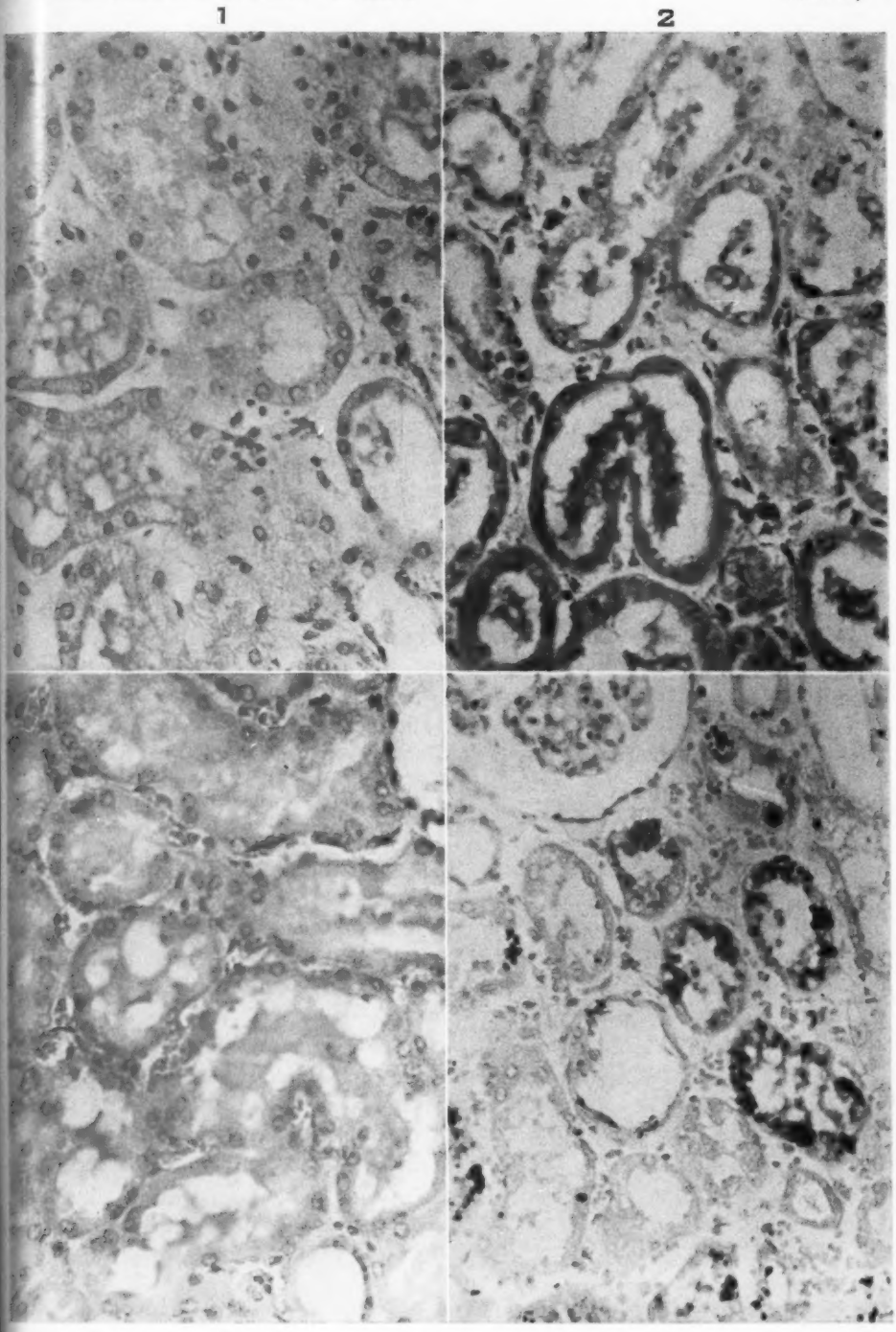
All sections were prepared by Gomori's method for the demonstration of iron.

FIG. 1. Kidney from case 9 showing amorphic masses of pale blue-reacting hemosiderin in lumina of the proximal convoluted tubules. × 433.

FIG. 2. Section of kidney from case 8 with diffuse free hemosiderin in the tubular lumina. × 433.

FIG. 3. Kidney, case 1. Pale blue pigment almost completely occludes the lumina. The pigmentation of the lining epithelial cells is marked by the counterstain. × 433.

FIG. 4. Case 2. The granular hemosiderin in the tubular epithelium may be a residue from previous intravascular episodes. The diffuse blue luminal pigment is thought to be a manifestation of the terminal intravascular hemolysis. × 433.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NUCLEAR STRUCTURE IN MAMMARY CARCINOMA *

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Study of the cytologic alterations in target organs in relation to fluctuations in sex hormones was stimulated by the descriptions of Papanicolaou[1] of the sexual cycle of the human female as revealed by the vaginal smear. The chromatic condensation that occurs in the nucleus of the vaginal epithelial cell under the influence of estrogens is now common knowledge. Nuclear alterations in the endometrial cells also are recognized and, as described by Gates and Warren,[2] the secretory stage of the menstrual cycle is characterized by the following changes: "The nucleus has become round rather than oval, stains less intensely than at other times, and most of the chromatin is collected near the nuclear membrane."

Although the architecture of the female breast has long been known to be under endocrine influence, there has been little study of the nuclear structure of the epithelial cells of a normal gland or of carcinomata arising therein. The growing data on hormone therapy in the treatment of mammary carcinoma have given indication that breast cancer cells are not truly autonomous but may be influenced by the estrogen and androgen levels of the host. The present investigation was undertaken to determine whether the cytologic appearance of breast carcinomata could be correlated with the endocrine status of the host. In addition, the survival time and metastases were evaluated to determine whether these could be correlated with the endocrine status of the patient.

METHODS AND RESULTS

In order to determine whether distinct cytologic patterns existed in the cells of mammary cancer, surgical and autopsy slides of such cases were chosen at random from the slide files, and the sections examined under oil immersion. The tissues had all been fixed routinely in formalin and stained with hematoxylin and eosin. It was soon evident that distinct nuclear types could be identified even if cytoplasmic features were not distinctive. For classification we have divided the nuclear types into five groups and indicated them as types 1, 2, 3, 4, and 5, the differentiation being based on the chromatin content and distribution. Since these changes are in the nature of a transition, cases which were not clear-cut were classified as intermediate; *vis.*, 2–3, or 1–2, etc. In

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no case was a mixture of types found other than with the type above or below it, and with a little experience reproducible identification of types was possible. The various types are depicted in Figures 1 to 5, while the criteria of classification are as follows:

Type 1. The nucleus stains readily and the chromatin is dispersed throughout the nucleus in distinct irregular cords. The nuclear membrane is distinct and stains well.

Type 2. The chromatin tends to be concentrated at the nuclear membrane, although a few condensed strands of chromatin may traverse what appears to be an achromatic sphere.

Type 3. The nuclear membrane appears delicate and is not sharply outlined. The chromatin is diffusely scattered throughout the nucleus in the form of very delicate strands. In addition there is a suggestion of an over-all basophilia of the karyolymph.

Type 4. The nucleus appears as a rather uniformly basophilic structure. The nuclear membrane is very thin and few or no chromatin strands are evident.

Type 5. There is condensation of basophilic material around the nuclear membrane, while within the nucleus vacuoles are present.

The data on the cases studied are collected in Tables I and II. Table I consists of cases in which the patients had received no endocrine therapy or castration, either surgically or by radiation therapy. In this group it is evident that a definite correlation exists between the nuclear types and the endocrine status of the patients as implied by their chronological age (Table III).

A similar correlation is found also when one compares the mean type with various age groups (Table IV).

Examination of Table II will show that of the 5 patients who received androgen and/or sterilization procedures, a progression in nuclear type occurred so that the mean type value was 3.4, although the mean age of the groups was only 40 years.

In 2 of the cases in this group, tissue was available for biopsy before and after androgen therapy and castration. The striking changes induced in the nuclear structure by these procedures is evident in Figures 1 and 4. Nuclear types 1 and 4 are from specimens for biopsy from the same case (R. B.) taken before, and 6 months after, oophorectomy and androgen therapy. Even more striking changes were evident in the case of E. L. whose original specimen was of a typical class 1 picture. The example of type 5 nucleus (Fig. 5) is from a biopsy specimen from the same patient taken 3 years after castration and following androgen therapy.

The reverse of these findings was noted in the case of B. S., an 80-year-old woman whose original biopsy specimen revealed type 4–5 nuclei (Fig. 6). The patient expired shortly after 1 month's treatment

TABLE I

Clinical Data and Results of Nuclear Typing for Patients Who Had Not Received Endocrine Therapy or Castration

Case	Age	Nuclear type	Survival	Metastases	Remarks
	yrs.		*mos.*		
M. B.	56	1	30	Lung, liver, adrenal, skeleton	Menopause 16 yrs. ago
M. D.	29	1	12*		Lactating breast
H. T.	42	1	18	Skeleton, pleura, lung, liver, ovary	
H. G.	49	1	9	Chest wall, lung, liver, mediosternum	Menopause 2 yrs. ago
F. P.	23	1	12	Lung, liver, sternum	
E. V. H.	49	1	12*		
A. D.	59	1	18	Lung, liver, retroperitoneum, spleen	
A. L.	47	1	48	Skin, lung, liver, spleen, skeleton, ovary	
M. C.	47	1	8	Skin, lung, skeleton	
C. O'G.	39	1	3*		
M. N.	43	1	3*		
A. S.	30	1	36	Lung, skeleton	
H. B.	56	1	12	Lung, ribs	Menopause 15 yrs. ago
J. D.	55	1	24		
V. D.	53	1	24	Lung	
E. M.	46	1	3		
R. W.	47	1	12		
K. J.	55	1	7	Lung, axilla, skeleton	
E. L.	31	1	48*	Skeleton, skin	
R. B.	41	1	30*	Skeleton, skin	
H. B.	38	1–2	3		
E. J.	35	1–2	48		
H. B.	59	1–2	14	Lung, skin, skeleton	
E. M.	46	1–2	3		
B. M.	70	2	24	Skeleton, skin	Menopause at 55 yrs.
F. J.	42	2	36		
M. J.	66	2	12*		Menopause 20 yrs. ago
K. M.	72	2	6	Lung	
R. D.	57	2	3*		
M. P.	55	2	12	Skin, skeleton	
B. M.	55	2	3*		
E. N.	61	2–3	11 yrs.	Lung, liver	
A. O.	61	2–3	72*	Lung, skeleton	Menopause at 51 yrs.
C. A.	53	2–3	24	Skeleton, skin	Menopause 9 yrs. ago
P. G.	64	2–3	12*	Axilla, skeleton	
J. B.	79	3	24*	Local skin and nodes	
A. B.	79	3–4	12*	Skin	Menopause at 35 yrs.
C. D. C.	58	3–4	3	Skeleton, lung	
B. S.	56	3–4	24*		
A. K.	70	4	60		Menopause at 40 yrs.
P. M.	71	4	48	Local spread	Menopause 26 yrs. ago
E. M.	58	4	15	Lung, ribs	Menopause at 53 yrs.
H. I.	63	4	60	Lung, skeleton	Menopause at 52 yrs.
M. R.	66	4	60*	Local spread, axilla	Menopause 13 yrs. ago
B. S.	80	4–5	36	Skin, lung	

* Still living.

with diethylstilbestrol. Sections taken at autopsy revealed a reversal to type 1–2 (Fig. 7).

Survival Time and Metastases

While no significant correlation appears to exist between the organs affected by metastatic spread and the age group of the patient, there

Table II

Clinical Data and Results of Nuclear Typing for Patients Who Received Endocrine Therapy and/or Castration

Case	Age	Nuclear type	Survival	Metastases	Remarks
	yrs.		*mos.*		
R. G.	46	4	24	Pleura, skin, lung	Radiation sterilization, and androgen therapy for 2 yrs.
E. L. 1945	31	1	48*	Skeleton, skin, ovary	Castration 3 yrs. ago, and androgens
1949	35	5			
Y. C.					
Jan. 1947	38	2–3	36	Pituitary body, stomach, liver, skeleton	Radiation sterilization, Nov. 1945
E. R. 1949	41	2	36*		Surgical castration, 1944
R. B.					
Jan. 1947	41	2	60*	Skin, ovary, skeleton	Resection of ovaries, June, 1947, plus androgen therapy
June, 1947		1			
Dec., 1947		3–4			
B. S.					
May, 1948	80	4–5	36	Skin, lung	Stilbestrol, May 14, 1948, to June 16, 1948

* Still alive.

Table III

Relation Between Nuclear Type and Mean Age

Nuclear type	No. of cases	Mean age
		yrs.
1	20	46
1–2	4	45
2, 2–3	11	59
3, 4, 5	10	68

Table IV

Correlation Between Mean Nuclear Type and Age Groups

Age group	No. of cases	Mean nuclear type	Percentage above minimum type (1)
To 55 yrs.	20	1.2	20
55 to 60 yrs.	12	1.9	90
60 yrs. and over	13	3.1	310

Intermediate types were given a numerical value halfway between the types, *viz.*, type 2–3 = 2.5.

does appear to be a correlation between the length of survival and the age of the patients. In the patients followed to death due to the carcinoma, those in the age group up to 55 had a 25-month mean survival

time; in the age group 60 years and older, the mean survival was 47 months. A mean survival time of 40.8 months was found for the 5 patients who had been castrated and/or had received androgen therapy, although the mean age of the group was only 40 years. The finding of an increased survival time in patients in the older age group is in accord with the observations of previous studies.[3]

Discussion

It is probable that the nuclear forms seen after formalin fixation and tissue preparation are artificial in that they may differ from the appearance in the living state. Nonetheless the artifacts, if such they be, are constant and indicative of a change in the colloidal structure of the nucleus. Studies of the fresh cells by mean of the phase microscope would certainly be desirable to elucidate this point further.

The observation that variations in nuclear structure occur in breast carcinoma cells in response to the endocrine status of the patient is additional support for the thesis of Nathanson,[4] who has indicated that breast cancer cannot be considered autonomous. The growing accumulation of data on the systemic components of the malignant state is in line with these observations and emphasizes that the prognosis and course of a malignant growth represent an expression of a tumor-host relationship. The findings reported here suggest that the cytologic appearance of the cancer cell may reflect some of the interplay of these factors in breast carcinomas. It would appear worth while to extend this type of observation to other cancers, both of target organs for the sex hormones and to those arising in tissues not usually considered to be influenced by hormonal control.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

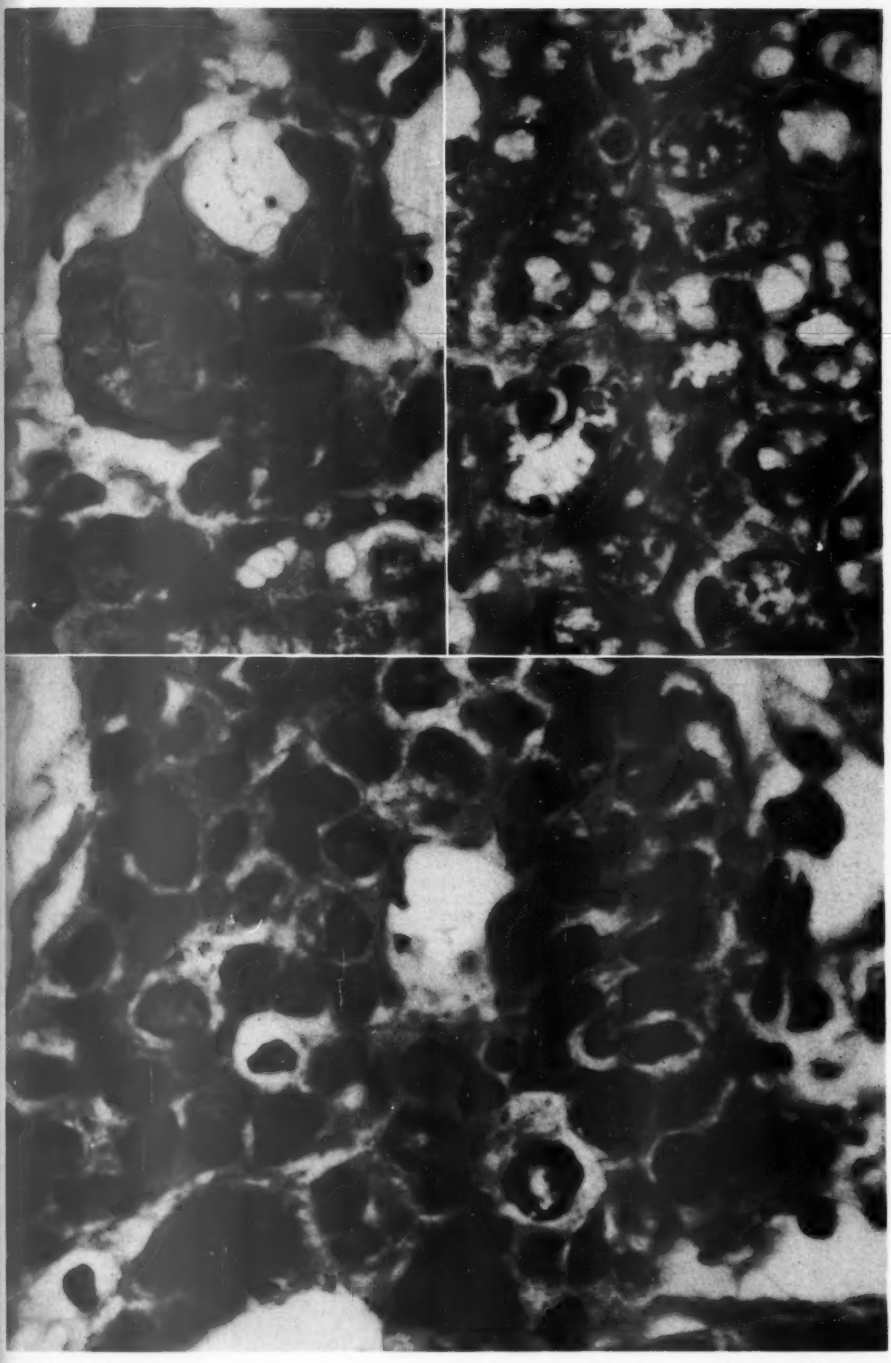
PLATE 128

FIG. 1. Type 1 nuclei, showing a well defined chromatin pattern.

FIG. 2. Type 2 nuclei, showing condensation of chromatin around nuclear membrane.

FIG. 3. Type 3 nuclei, showing a delicate lacy chromatin pattern.

Xylo



Black and Speer Nuclear Structure in Mammary Carcinoma

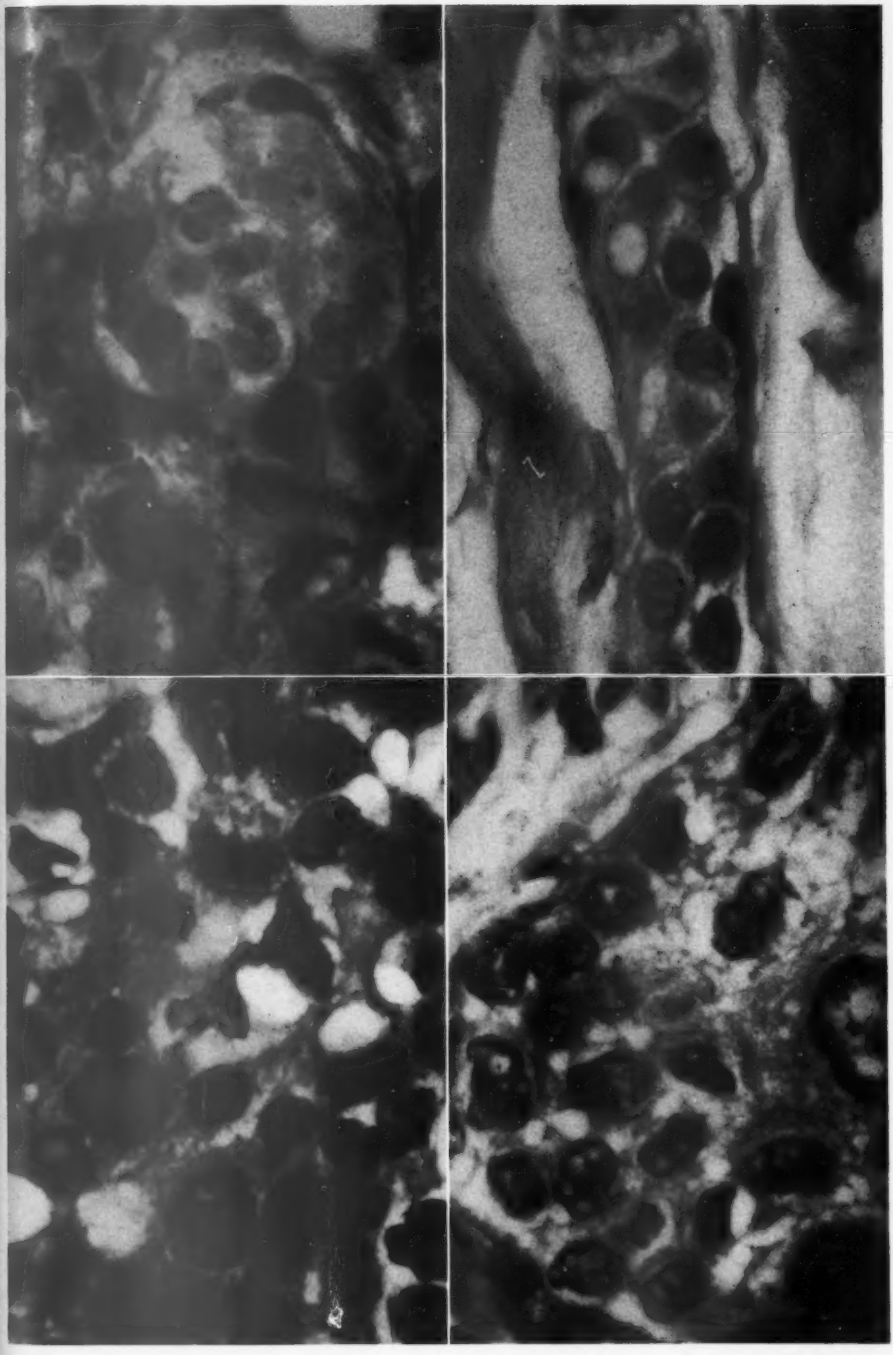
PLATE 129

FIG. 4. Type 4 nuclei, showing lack of distinct nuclear chromatin and diffuse basophilia. This specimen was obtained for biopsy from the same patient as Figure 1 but after castration and androgen therapy.

FIG. 5. Type 5 nuclei, showing vacuolization. This tissue was obtained after castration and androgen therapy. The original biopsy specimen showed typical type 1 nuclei.

FIG. 6. Type 4–5 nuclei, from breast carcinoma in an 80-year-old woman.

FIG. 7. Type 1–2 nuclei, from the same case as Figure 6, but after 1 month's treatment with diethylstilbestrol.



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7

Black and Speer Nuclear Structure in Mammary Carcinoma

THE EFFECT OF SODIUM SULFADIAZINE ON THE RENAL TUBULE (NEPHRON) OF THE ALBINO RAT *

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The advent of the sulfa drugs in the early thirties was followed by their widespread and often indiscriminate use in the treatment of infectious diseases. Such use resulted in toxic effects which were described in due course in numerous reports. Also, studies were undertaken to determine the specific alterations responsible for these effects. Renal excretion of the sulfa drugs and possible renal damage caused by them soon occupied the attention of certain investigators. Varying degrees of renal damage have been described with emphasis sometimes on glomerular changes, sometimes on damage of the tubules, and again on injury to the renal vessels and interstitial tissue.

Murphy, Kuzma, Polley, and Grill,[1] in clinicopathologic studies, considered three types of renal damage resulting from the use of the sulfonamide compounds, of which one was a simple type due to their toxicity and associated with degeneration and obstruction of the tubule accompanied by glomerular changes. The latter were described as being similar to those found in diabetic patients, consisting of intracapillary glomerulosclerosis with glomerular hyalinization. In all of the kidneys studied by them, regardless of other renal changes, these investigators found variable degeneration of the tubules which they considered as representing degrees in the severity of one process rather than a difference in kind of response. Also, injury leading to disintegration of renal vessels was said to be indicated by perivascular cellular infiltration and multinucleated giant cells.[1,2] Afferent arterioles were described as being thickened and hyalinized.[2,3]

The majority of investigators have emphasized changes in the tubules brought about by toxic doses of the sulfonamide drugs. Perhaps the most comprehensive report is that by Lehr and Antopol[4] who, in describing damage of the nephron caused by sulfadiazine, employed the term "calcifying nephrosis" to indicate the type of lesion produced. They, as well as we, used albino rats in their experiments. On gross examination of the kidneys of their rats, Lehr and Antopol noted two whitish streaks extending "concentrically on the cut surface" of the kidney which, by means of the von Kossa phosphate stain, they identified as being due to "calcified renal tubules" and as being "distal convoluted tubules, Henle loops and collecting tubules." In further studies

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involving the use of acetylsulfadiazine they found damage of a somewhat different type, described as being "severe parenchymatous and fatty degeneration of the renal tubules with little calcification." Endicott and Kornberg,[5] using sulfadiazine in experiments on albino rats, concluded that "the collecting and distal convoluted tubules appear to be the chief sites of damage." But Maisel, McSwain, and Glenn,[2] in studying the pathologic reactions produced in dogs by the action of sodium sulfadiazine, found damage in both proximal and distal convolutions of the nephron as well as in the collecting ducts. They mentioned pyknotic nuclei and a swelling of the cells of these convolutions accompanied by indistinct cell outlines. They also observed mitotic figures in the cells of certain tubules.

Interest in the toxicity of the sulfonamide drugs has been revived because of the possible relationship of such toxicity to the clinical syndrome designated by Lucké[6] as "lower nephron nephrosis"—a term which is neither anatomically nor topographically applicable. In analyzing 538 fatal cases of this syndrome as variously produced, Lucké found in 67 cases that there was no predominant etiologic factor other than sulfonamide intoxication. In all 538 cases, comparable damage was located in the "lower nephron," a portion of the nephron consisting of the ascending limb of Henle's loop and the distal convolution. Such damage was described as varying from slight degeneration to actual necrosis or complete degeneration of this portion.

Despite the large amount of work done to determine the localization and extent of damage of the nephron, such damage has not been further specified in relevant papers read by us. There have been suggestions of specific localization of damage. For example, Luetscher and Blackman,[7] in reporting clinical cases of sulfathiazole intoxication, stated that "the renal lesions found in two cases suggest that the dissociation of salt and water excretion may be related to changes in certain specific portions of the tubule."

This paper deals with certain renal effects resulting from the action of sodium sulfadiazine only. The experiments and study were designed to make possible the exact location of the site or sites of damage within the nephron and to determine whether such damage was accompanied by fatty degeneration.

Material and Methods

Albino rats of both sexes were used. They weighed from 175 to 340 gm. and were kept in metabolism cages, fed a balanced diet, and had access to water at all times. Sodium sulfadiazine was selected because of its solubility and easy handling as compared with the free compound.

The varied doses used were each dissolved in 2 cc. of sterile distilled water and injected intraperitoneally. In 5 of 14 rats used, a single dose of 1.7 gm. per kg. of body weight was injected. This amount corresponds to 1.5 gm. of the free sulfadiazine per kg. of body weight and is equivalent to Lehr and Antopol's[4] LD_{60}. For other doses used, see Table I.

In Table I, it will be noted that approximately one-third of the treated rats died. Their kidneys were removed as promptly as possible

TABLE I

Data on the Albino Rats Used in the Experiments Described

Series no.	Rat no.	Weight	Dose per kg.	Actual dose	Number of injections	Died, killed
		gm.	gm.	gm.		hrs.
II	0	290	Control	0.0	0	48 k
	1	260	Water	2 cc.	1	48 k
	2	290	3.4	0.986	1	24 k
	3	270	3.4	0.918	1	18 d
	4	290	1.7	0.493	1	24 k
	5	270	1.7	0.459	1	60 d
III	1	175	0.85	0.149	4	96 k
	2	290	0.85	0.246	4	75 d
	3	305	0.85	0.259	6	110 d
	4	295	0.57	0.167	5	96 k
	5	340	0.57	0.193	7	136 k
	6	230	Water	2 cc.	8	180 k
IV	1	280	1.7	0.476	1	48 d
	2	230	1.7	0.391	1	48 k
	3	270	0.85	0.230	4	80 k
	4	290	0.85	0.247	4	80 k
V	1	255	1.7	0.434	1	45 k

and handled as were those of the rats that were killed at certain intervals in order to correlate the location and extent of damage of the tubule. Controls of two types were used. The animals of one type received no injections and those of the other received injections of sterile distilled water in amounts equivalent to those given rats receiving the sodium sulfadiazine. In those rats given more than one injection, the interval between injections was 24 hours. Of the animals killed, some were anesthetized with ether and some by the intraperitoneal injection of a 10 per cent solution of dial-urethane in amounts of 0.6 cc. per kg. of body weight. While anesthetized, the abdominal cavity was opened, its contents examined, and the kidneys excised. In some instances, they were first injected with India ink in order to reveal aspects of the renal vessels and their possible damage.

In view of the conflicting reports, it seemed that the site and extent of damage within the nephron could be determined only by studying isolated nephrons in correlation with serial sections. Upon excision of

the kidneys, each was divided into two portions. One portion was fixed in either Bouin's fluid or in 10 per cent formalin preliminary to further histologic treatment. The other was placed for maceration in a 20 per cent solution of HCl. The portion reserved for histologic study was divided into two pieces: one was sectioned serially at 8 μ and stained with Goldner's[8] modification of Masson's trichrome stain; the other was sectioned at 10 μ on the freezing microtome and stained for the presence of fat. In staining for the latter it was necessary to distinguish mitochondrial (interliposomal) fat from abnormal fat deposits or degeneration products. For this purpose sudan black, sudan orange in glycerine, and scharlach R (sudan IV)[9] were used tentatively. Sudan IV was found most suitable and accordingly was used in all tests for fat since only neutral fat was stained and it was possible to distinguish between abnormal fat particles, mitochondrial fat, and the homogeneous fat of the cytoplasm. Sections stained for fat were counterstained with Mayer's hematoxylin in order to reveal possible degenerative changes in the nuclei.

Pieces of kidney to be macerated were cut 3 mm. thick and placed in sudan IV for 2 to 4 hours, transferred to the HCl solution for 15 to 20 hours, and then placed in alkalinized water until the tubules could be isolated readily. The preliminary staining in sudan IV served to prevent the development of an artificial fatty change due to the acid. The tubules, isolated with glass needles, were transferred to glass slides and mounted in glychrogel.[10] Thus, the tubules and especially the segments containing fat could be measured and studied microscopically under low or high power objectives. It was not possible to stain isolated tubules so as to permit accurate observation of cytologic changes characteristic of the various segmental lesions within the tubule. For this a study of the histologic sections was necessary.

Results of Experiments

The Occurrence of Fat

In Table II a summary is given of the distribution of fat in the cells of various portions of the nephron as a result of the action of sodium sulfadiazine. This summary was made possible by studying frozen sections of the kidney and isolated nephrons, both stained with sudan IV.

Rats comprising the acute series in Table II were given only one injection of the drug as follows: 2 rats were given the massive dose of 3.4 gm. and 5 were given one-half this dose. The fat content of the nephrons in these rats was related more to *time* than to dosage. In the kidney of one rat given 3.4 gm. and dying at the end of 18 hours, fat occurred in the cells of the same segments of the nephron (see below) as

TABLE II

Occurrence and Distribution of Fat in the Kidneys of Albino Rats

Time	Proximal convolution in thirds			Ascending limb		Distal convolution		Collecting ducts
	Upper	Middle	Lower	Lower half	Upper half	First half	Second half	
Acute series								
hrs. 18			Fine, uniform	Fine, uniform			Fine, peripheral	Fine, uniform, peripheral
24	Patchy,* peripheral	Patchy, peripheral	Massive, uniform	Massive, uniform		Patchy, peripheral	Massive, peripheral	Fine, uniform
48	Massive, peripheral	Patchy, peripheral	Massive, uniform	Massive, uniform	Fine, scattered	Fine, peripheral	Massive, peripheral	Fine, massive in papillary ducts and calyx
Chronic series								
75–136			Massive, uniform	Massive, uniform			Massive, peripheral	Massive, uniform to upper calyx

* Patchy means occurring in alternating groups of cells.

contained it in all experiments, namely, the terminal third of the proximal convolution, the first half of the ascending limb of the loop of Henle, and the second half of the distal convolution. In the kidney of 2 rats given, respectively, 3.4 and 1.7 gm. of the drug, and killed at the end of 24 hours, fat was variably present in the cells of both convolutions but was typically present in those of the first half of the ascending limb of Henle's loop (Table II, 24 hours). In the kidneys of 4 rats given only 1.7 gm. of the drug and killed at the end of 48 hours, fat occurred to some extent in the cells of all subdivisions of the nephron except in those of the thin segment (Table II and Figs. 1, 4, 7, 9, 11, and 13).

Rats comprising the chronic series of Table II were given, at 24-hour intervals, from 4 to 8 injections of the drug. Two of these were given 0.57 gm. 5 and 7 times, respectively, and 5 were given 0.85 gm. Of the latter, one rat

was given this dose 6 times; the others, 4 times. The fat content of the nephrons of these rats is related more to *dosage* than to time. Fat occurred chiefly or only in the cells of definite segments of the nephron (see above, rat given 3.4 gm. and dying at the end of 18 hours), namely, the terminal third of the proximal convolution (Fig. 7), the first half of the ascending limb of the loop of Henle (Fig. 9), and the second half of the distal convolution (Fig. 13).

The cells of the middle third of the proximal convolution were peculiar. In this third, regardless of time and dosage, groups of cells contained fat while adjacent groups did not, thus producing in isolated nephrons a patchy appearance (Fig. 4, upper two-thirds of the larger segment). A similar condition was seen in the cells of the first half of the distal convolution, but only at the end of 24 hours in the kidney of a rat given a single large dose of the drug. In another rat given an equivalent dose but killed at the end of 48 hours, all of the cells of this half contained fat. In general, the fat content in the cells of various portions of the nephron attained a maximum by the end of 48 hours.

Because of the factors of time and dosage, the nephron in all experiments was subdivisible into specific segments in regard to its fat-containing portions. In some experiments, there was a more extensive involvement in addition to these segments. The segments suggested specific reaction sites within the nephron. Especially noteworthy was the invariable occurrence of large amounts of fat in the cells of the first half of the ascending limb of Henle's loop. No part of this limb has been shown with certainty to possess properties of functional or pathologic significance. The cells of the thin segment alone in all experiments and, with a minor exception, those of the second half of the ascending limb of Henle's loop, did not contain appreciable amounts of fat or appear to be damaged. In the cells of the thin segment, this was not necessarily contingent upon their thinness. In the cells of the convoluted portions of the nephron, fat, when present, was concentrated in a layer adjacent to the basement membrane (peripheral, Table II). This was in contrast to a more uniform distribution in the cells of the non-convoluted, terminal portion of the proximal convolution and in those of the first half of the ascending limb of Henle's loop (Figs. 7 and 9). The cells of the collecting ducts contained fat at times and variably. It commonly was present in considerable amounts in the cells of the papillary ducts and in the epithelium of the upper portion of the calyx.

The presence of fat in the cells of the involved segments of the nephron as designated above, suggests that it is released *in situ* following

some change in cell protein caused by the action of the drug and is, therefore, a fatty degeneration (phanerosis). It also suggests that the nephron contains more reactive sites than are indicated by its anatomical subdivisions and, indeed, more than have been functionally demonstrated.

The Sites and the Extent of Damage

It was evident from serial sections that cell damage in portions of the nephron was coextensive with the portions observed to contain fat in the isolated nephrons. Such damage was indicated in certain segments (Figs. 2, 3, 6, 8, and 14) and transitions from damaged to relatively undamaged segments were evident (Figs. 5, 10, and 12). In the kidneys of rats given a single dose of 3.4 or 1.7 gm. of the drug, injury, like the fat content of the nephron, was greater and more extensive. In the damaged portions of the nephron, the cells showed vacuolated cytoplasm and variably swollen nuclei with chromatin undergoing dissolution. Pale-staining globules of various sizes commonly were present in the glomerular capsule and in the lumina of the nephrons. They were components of a web-like, obstructing detritus. The basement membrane occasionally was ruptured as a part of the injury. Within 48 hours cell damage was maximal. In the proximal convolution, such damage was indicated in its first third by the presence of small vacuoles (Fig. 2) and slightly altered nuclei; in its middle third, by alternating groups of damaged and relatively undamaged cells (Fig. 3), and in its terminal third, by a general cell disorganization (Fig. 6). The cells of this third in the kidneys of rats given repeated, small doses of the drug became progressively squamous by the loss of cytoplasm until the nuclei were flattened against the basement membrane. It appeared that the cytoplasm of such cells disintegrated in a wave-like progression along their luminal surfaces until often none remained to contain the nuclei which then floated away or disappeared, leaving only the basement membrane. Damage in the first half of the ascending limb of Henle's loop and the second half of the distal convolution was indicated by vacuolated cells accompanied by cytoplasmic and nuclear degeneration in which the vacuolation often was obscured (Figs. 8, 10, and 12).

In the kidneys of those rats given a single dose of 1.7 gm. of the drug (Lehr and Antopol's[4] LD_{60}) and in those given repeated, smaller doses, dilatation of the nephrons and ducts extended from the middle third of the proximal convolution to the end of the collecting duct system. A similar dilatation was observed by Endicott and Kornberg.[5] This may be due to casts of mixed composition which in some instances filled the

lumina. The nephrons of rats given repeated, small doses of the drug contained the usual damaged segments but the damage varied from moderate to severe depending on the length of time the rat lived.

Summary

1. Fourteen albino rats weighing from 175 to 340 gm. were injected intraperitoneally with sodium sulfadiazine varying in amounts from 0.57 to 3.4 gm. per kg. of body weight. Seven of these were given a single injection each and 7 were given multiple injections. Appropriate controls were used.

2. Approximately one-third of the treated rats died and the remainder were killed at intervals in order to determine the site and extent of renal damage in relation to both time and dosage. Parts of the same kidney were used to obtain frozen sections while others were macerated and microdissected to obtain isolated nephrons. Both were stained with sudan IV. The results may be summarized as follows:

(a) When sodium sulfadiazine is injected into rats in single, large doses, fat appears within 24 to 48 hours in some of the cells of all subdivisions of the nephron except the thin segment. With repeated, smaller doses a maximal content of fat occurs chiefly or only in the cells of specific segments of the nephron. These are the lower third of the proximal convolution, the first half of the ascending limb of the loop of Henle, and the second half of the distal convolution. Transitions between segments with and without fat are sharp.

(b) The occurrence of fat in the cells of the nephron is variable, depending on time and dosage. In rats given the massive dose of 3.4 gm. of the drug per kg. and killed at the end of 24 hours, alternating groups of cells with and without fat occur in the first two-thirds of the proximal convolution, and in the first half of the distal convolution, but all cells contain fat in the remainder of these convolutions. When one-half of this dose is given 48 hours before death, fat is present in all cells of the distal convolution but in much less quantity in its first half, and there are alternating groups of cells with and without fat in the middle third of the proximal convolution. Also, a scant content of fat occurs in the cells of the second half of the ascending limb of the loop of Henle in addition to the large content invariably present in the cells of the first half of this limb.

3. Microscopic study of serial sections of the kidneys of treated rats reveals damaged portions of nephrons which are coextensive with those containing fat. The chief aspect of such damage consists initially of vacuolated cells with variably degenerating nuclei. Damage is maximal within 48 hours in the kidneys of rats given single, large doses of the

drug. With variations in dosage and a survival time ranging from 75 to 136 hours, damage consists of complete necrosis of segments within each of the anatomical subdivisions of the nephron except the thin segment.

4. The segmental effect of sodium sulfadiazine, as indicated, suggests that the nephron contains reactive sites in excess of its anatomical subdivisions and that these sites normally may serve specific functions in the formation of urine.

5. The fat content and damage of the cells of the collecting duct system are not localized in segments. This is believed to indicate a relatively non-specific effect of the drug on the cells of this system.

6. The kidneys of rats used as controls contained no visible fat or evidence of cell damage.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

All photomicrographs are of portions of isolated or sectioned nephrons of rats' kidneys and are referable to Table I. Black particles visible in the isolated portions of nephrons indicate the presence of fat.

PLATE 130

FIG. 1. Rat 1, series V, killed at the end of 45 hours. Glomerulus and upper portion of an isolated proximal convolution. Black stipple indicates the presence of fat droplets, but not in the glomerulus. In the latter it is the result of the injection of India ink prior to death in order to reveal aspects of the renal circulation. × 105.

FIG. 2. Glomerulus and tubular outlet in histologic section showing small, light vacuoles in the cytoplasm of the cells of the outlet. These indicate the sites of dissolved fat. The section was made from the same kidney used to obtain the isolated portion of the nephron in Figure 1. × 210.

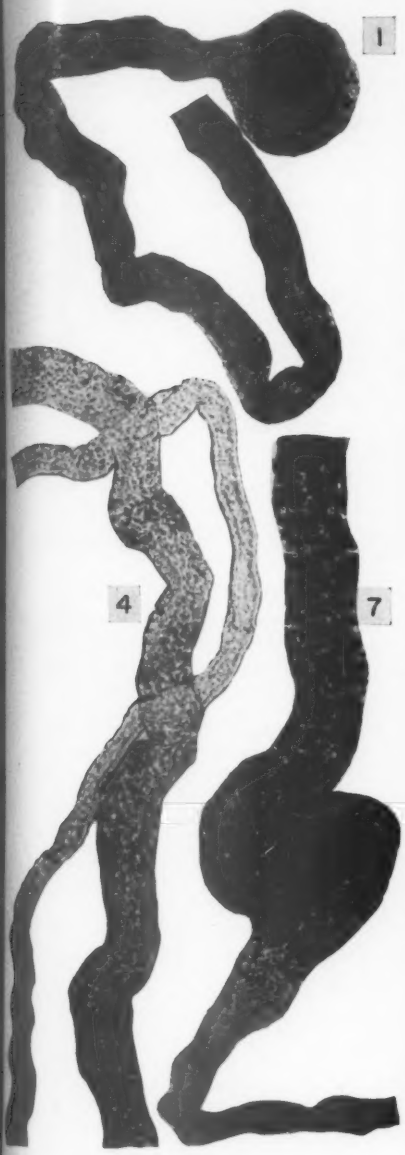
FIG. 3. Middle portion of a proximal convolution obtained from the kidney of rat 5, series II, which died at the end of 60 hours. The epithelium of this portion of the right wall is markedly damaged while that of the left wall is not. Damage of this irregular, patchy type occurred in this portion in all experiments. × 150.

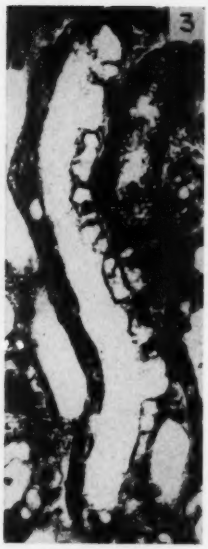
FIG. 4. Rat 3, series III, which died at the end of 110 hours. Isolated segment of the lower portion of the middle third of a proximal convolution (larger segment) and likewise of an ascending limb of the loop of Henle (smaller segment). The topmost part of the larger segment is almost clear, but is succeeded downward by three fairly distinct, stippled patches and a terminal, more heavily stippled portion. Such stippled areas indicate the presence of fat and coincide with the variably damaged epithelium shown in Figure 3. × 120.

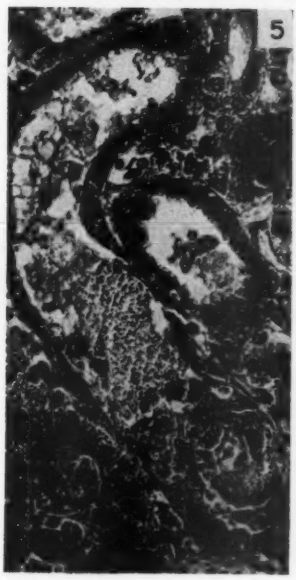
FIG. 5. Histologic section of the junction between the middle and lower thirds of the proximal convolution, comparable to that portion of the nephron shown at the bend of the lower part of the larger segment in Figure 4. Of note are the relatively undamaged upper and necrotic lower portions of the curved segment. Such junctions between undamaged and damaged parts are related to the greater content of fat in the necrotic part. × 150.

FIG. 6. Rat 4, series IV, killed at the end of 80 hours. Terminal portion (descending limb) of a proximal convolution shown in continuity with the first part of a thin segment. The cellular damage coincides with the massive content of fat as indicated by the black particles in Figure 7. × 120.

FIG. 7. An isolated, terminal portion of a proximal convolution like that shown in section in Figure 6 and from the kidney of the same rat. The lower clear portion (thin segment) is almost free of fat. × 150.







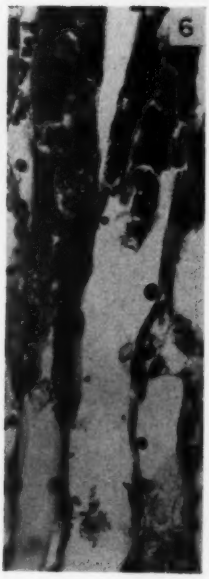


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PLATE 131

FIG. 8. Rat 2, series II, killed at the end of 24 hours. The initial medullary portion of an ascending limb of Henle's loop. The damaged epithelium is coextensive with its fat content, as shown in Figure 9. × 195.

FIG. 9. Rat 2, series IV, killed at the end of 48 hours. An isolated portion of a nephron like that shown sectioned in Figure 8. × 155.

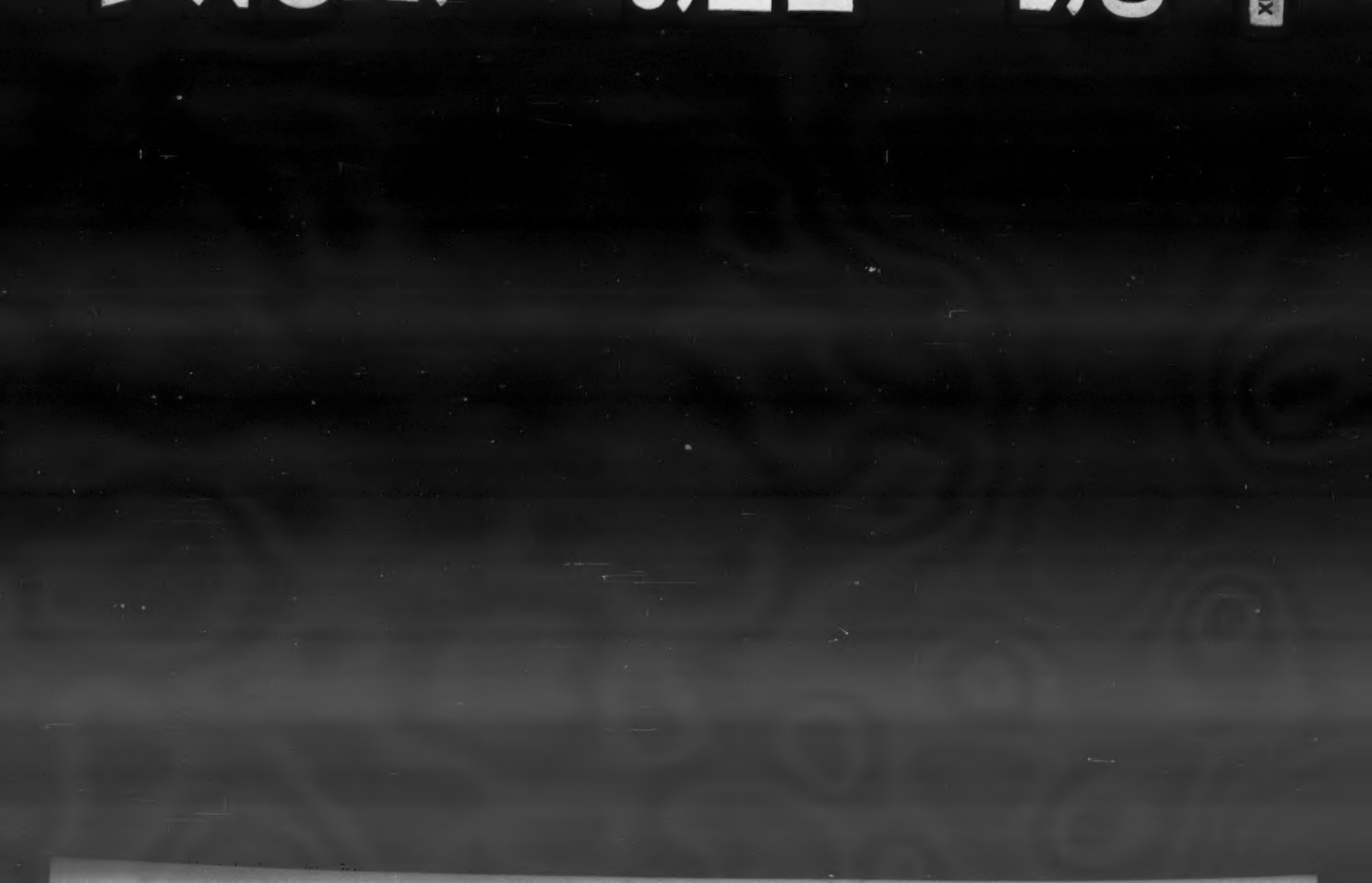
FIG. 10. Rat 3, series II, which died at the end of 75 hours. Segment of the middle portion of the ascending limb of Henle's loop showing marked and less marked damage in the lower and upper halves, respectively. Such damage coincides with the variable presence of fat as shown in the lower and upper halves of Figure 11. × 195.

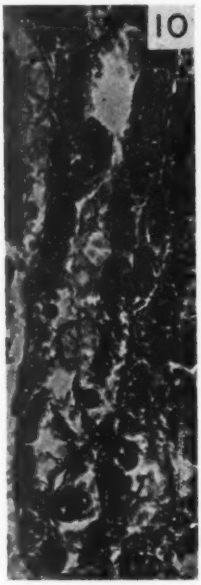
FIG. 11. Segment of the middle portion of an isolated ascending limb of Henle's loop showing the transition between its lower and upper halves in respect to its fat content (many to few black particles), from the same rat as used for Figure 10. × 155.

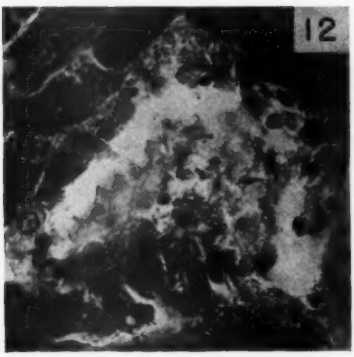
FIG. 12. Rat 4, series IV, killed at the end of 80 hours. Section of the region between the first and second halves of the distal convolution showing slight to marked damage in its left and right portions, respectively. Such damage corresponds to the portions containing large and small amounts of fat as indicated by black particles in Figure 13. × 235.

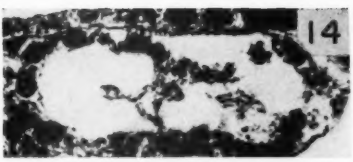
FIG. 13. Segment of a distal convolution showing, left to right, many to relatively few black particles. This is typical of the transition from a greater to a lesser content of fat which is present at the junction between its first and second halves. This is from the same rat and is the same portion of a convolution as indicated in Figure 12. × 195.

FIG. 14. Rats 2 and 4, series II, killed at the end of 24 hours. Section of a portion of the distal convolution showing, in its upper and lower parts, variable damage of its epithelium. Damage of this type occurs in the first half of this convolution and in the upper two-thirds of the proximal convolution, when a single, large dose of the drug is given and the rat is killed at the end of 24 hours. × 195.









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OBSERVATIONS ON THE MITOTIC REACTION INDUCED IN THE LIVERS OF RATS BY THIOUREA*

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In the course of our studies on the toxic effect of thiourea on rats, we have observed that this compound, given in large repeated doses, causes the appearance of numerous mitotic figures in the liver. This phenomenon, which has already been reported in a preliminary note,[1] will be described in detail and discussed in the present communication.

Young albino rats of both sexes, weighing 70 to 100 gm., were used. Thiourea in 10 per cent aqueous solution was administered by the intraperitoneal route in daily injections. From a total of 105 rats, 71 received 1 to 14 injections of 0.4 gm. of thiourea and the remaining 34 rats received a varying number of injections of 0.3, 0.2, 0.1, and 0.05 gm. of thiourea. The livers, removed immediately after death, were fixed in Carnoy's and Zenker's fluids and embedded in celloidin-paraffin. Sections 5 μ in thickness were stained with hematoxylin and eosin and with Heidenhain's iron hematoxylin.

RESULTS

Most of the observations were made on rats treated on 3 successive days with 0.4 gm. of thiourea. These doses proved to be highly toxic. Approximately 20 per cent of the rats succumbed after 1 or 2 injections. Of 41 rats which survived to receive 3 daily injections of 0.4 gm. of thiourea, 12 succumbed and 29 were sacrificed several hours after the third injection.

The gross appearance of the livers was not altered significantly. On microscopic examination the only changes were in the central parts of the lobules and consisted of congestion of the sinusoidal capillaries and of slight hydropic vacuolization of the liver cells. Occasionally, slight alterations of the lining of the central veins were seen. Widening of the pericapillary spaces was observed, but only rarely. The general texture of the liver remained unchanged. In no instance was there necrosis of hepatic tissue. The liver cells showed no deposition of fat, and they were often rich in glycogen.

Mitotic figures were present in the livers of nearly all 41 rats receiving this treatment, but varied in number from animal to animal. In 10

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of the treated rats the number of such figures was very high, each microscopic field containing 12 to 18.* In 8 rats they were numerous, each field containing 6 to 12 mitotic figures. A moderate number was found in 7 of the rats, 1 to 6 per microscopic field. In 13 of the experimental animals the number of mitotic figures was small, one being found in 1 to 3 microscopic fields, and in 3 rats none was found. The animals which showed no figures were examined 48 to 50 hours after the first injection, at a time when mitotic figures are just beginning to appear. The distribution of mitotic figures in different areas of the liver was not uniform. They were dispersed throughout the entire lobule, but seemed to be fewer around the central vein than in the midzonal and peripheral parts.

The mitotic figures in the livers of rats treated with thiourea were mostly of normal appearance and all phases of cellular division were present (Fig. 1). No special preference for a particular phase was observed. In some livers all mitotic figures, or a part of them, presented abnormal features. The main alteration was the scattering of chromosomes (Fig. 2). The individual chromosomes in the form of minute roundish or ovoid granules were dispersed uniformly throughout the cell body. They appeared dark blue with hematoxylin staining. At times the chromosomes were not entirely uniform in size and side by side with minute dark chromosomes there were others somewhat larger and of drop-like appearance (Fig. 3). Many chromosomes were situated in pairs in diplococcus-like fashion (Fig. 4). In other instances the chromosomes were not evenly distributed throughout the cytoplasm but were concentrated in the midzonal part of the cell and arranged in the form of a sphere, leaving the central zone completely free (Fig. 5). There were also cells in which the chromosomes were scattered only in the central area. The scattering resulted from alterations occurring in the late prophase, and all transitional steps from entirely normal late prophase to cells with scattered chromosomes in all degrees of chromosomic dispersion could be noted.

Many cells with abnormal mitotic figures presented more complex features. These were cells in which spindles were formed and some chromosomes were arranged in a metaphase plate, while the rest were scattered either in the vicinity of the plate or at some distance (Fig. 6). It appeared that the cells with partly dispersed chromosomes or with chromosomes concentrated in the central area of the cell completed the process of division. The subsequent history of the cells with the widely scattered chromosomes is of particular interest. They became multinucleated and contained up to 7 small nuclei (Fig. 7). Apparently each

* The mitotic counts were made in microscopic fields of 0.4 mm. in diameter.

small nucleus was formed from a single chromosome. The nuclei were minute but of completely regular structure except for the plasmosome which might be present but usually was absent. The nuclei originating from individual chromosomes formed nuclear aggregates, in which the individual nuclei could be discerned. The minute nuclei might fuse and form large lobated nuclei sometimes resembling the nuclei of megakaryocytes (Fig. 8).

The population of liver cells in rats treated with thiourea presented a picture of considerable diversity, showing great differences in the size of individual nuclei and numerous binucleated and multinucleated cells.

Mitotic figures were present also in the livers of rats which received 2 injections of 0.4 gm. of thiourea and succumbed, or were sacrificed 48 hours after the beginning of the experiment. When only one dose of 0.4 gm. of thiourea was administered, a definite mitotic response was observed after 55 hours. On the other hand, in the livers of 9 rats which succumbed or were sacrificed 24 hours after one injection of 0.5 gm. of thiourea, no mitotic figures were found. This shows that the time elapsing after the first injection is an important factor in the appearance of mitotic figures. A single dose which is ineffective after 24 hours causes mitotic figures after 55 hours. There were no mitotic figures in the livers of rats which were treated with 0.4 gm. of thiourea on 5, 9, 10, 12, 15, and 16 successive days. The period of mitotic activity in the rats treated with repeated doses of 0.4 gm. of thiourea is thus limited. The mitotic figures appeared within 48 to 76 hours after the first injection irrespective of the total dose given. The peak of the mitotic wave was found at 53 to 56 hours after the first injection.

In investigating the effect of small doses of thiourea on the mitotic activity in the liver, 4 rats received 0.3 gm. of thiourea on 3 successive days. In these rats numerous mitotic figures were seen 52 hours after the first injection and fewer after 72 hours. Twelve rats receiving 3 injections of 0.2 gm. of thiourea on 3 successive days behaved in the same way, namely, numerous mitotic figures were found 52 to 53 hours after the first injection and fewer after 70 hours. Mitotic figures were not found after 4 and 5 injections of the same dose 96 and 120 hours after the first injection. The effect of smaller doses, such as 0.1 and 0.05, was definitely less pronounced. Few mitotic figures were observed in the livers of 5 of 10 animals treated on 3 and 4 successive days with 0.1 gm. of thiourea 52 and 72 hours after the first injection. In the other 5 rats none occurred. Of 4 animals receiving 0.05 gm. of thiourea on 3 successive days, 3 showed no mitotic figures and in one only a few were found.

Discussion

Mitotic figures are occasionally seen in the adult liver but they are very rare (Pfuhl[2,3]). According to Brues and Marble[4] and to Dawson,[5] one mitotic figure in 10,000 to 20,000 cells may be expected in the liver of the adult rodent. Mitotic figures in the liver cells of young growing rats are more common. Nevertheless, their number is small and on routine examinations of liver sections they can be found only occasionally. In spite of this, there is no doubt that the liver cells possess remarkable ability to multiply. It is common knowledge that after extirpation of large parts the liver regenerates completely in a very short time. It is highly probable that karyokinesis is the chief mechanism in the restoration of the organ. Indeed, 24 hours after the removal of parts of the liver, numerous mitotic figures appear in the remaining hepatic tissue. They are also a common feature in livers undergoing necrobiotic changes resulting from exogenous or endogenous noxae.

In the present paper it is shown that numerous mitotic figures appear in the livers of rats treated with thiourea. It is important to note that in this case they are not associated with any loss of liver parenchyma. The microscopic examination of these livers never revealed necrotic changes. The only alterations were slight or moderate hydropic vacuolization of the hepatic cells and slight damage to the lining of the central veins and sinusoidal capillaries. Even these minor changes were not constant and many livers with numerous mitotic figures failed to disclose any structural abnormalities. The marked mitotic activity which takes place in the livers of rats treated with thiourea therefore cannot be regarded as an attempt to replace lost hepatic tissue.

The observations on the occurrence of mitotic figures in the livers of rats treated with thiourea is not without parallel. A number of authors working with a variety of substances of totally diverse origin described mitotic figures in the liver which, like the mitotic figures in thiourea poisoning, were not associated with visible hepatic damage. De Walsche[6] observed very large numbers of mitotic figures in the livers of 2 rats injected with staphylococcus toxin. Mayer[7] reported the presence of numerous mitotic figures in the livers of 2 mice after the injection of 1 cc. of a 1 per cent solution of trypaflavine. He emphasized the absence of liver damage except for a slight vacuolization of the liver cells. Pfuhl[3] found mitotic figures in the livers of guinea-pigs killed after one injection of trypan blue. He saw no evidence of cellular degeneration in the livers of the treated animals. Deane[8] also found abundant mitotic figures in all stages in mice receiving trypan blue. The alterations in the hepatic cells were slight and limited mainly to a

reduction of the glycogen content and minor deviation in the structure of mitochondria.

A similar effect on the liver not accompanied by parenchymal damage has been observed also after injection of different tissue extracts, especially of homologous liver. McJunkin and Breuhaus[9] injected homologous crushed liver into the peritoneal cavity of adult rats and after 2 injections (7 to 9 days) found a small number of mitotic figures in the liver. One or 2 injections of macerated liver into partially hepatectomized rats provoked mitotic figures in considerable numbers, the mitotic activity being much more pronounced than in untreated, partially hepatectomized rats.

The recent work of Wilson and Leduc[10] on mice is in accord with the above findings. These authors observed an increased number of mitotic figures in young and adult mice after the injection of homologous pulped liver, autolyzed liver, kidney, guinea-pig liver, boiled mouse liver, and boiled egg yolk.

Human pathology also offers examples of mitotically active livers in a variety of conditions not necessarily associated with necrobiosis. Mallory[11] mentioned having seen mitotic figures in the human liver in a great variety of pathologic conditions, and even in one case where the liver showed no visible morphologic changes. MacMahon[12] observed the presence of mitotic figures in adult human liver. There were only slight changes in the parenchyma such as fatty infiltration and dissociation of the liver cells in the center of the lobule, and inconspicuous bile stasis. No necrobiosis was found. The author concluded that liver regeneration is independent of loss of parenchyma and of the presence of necrotic material in the liver tissue. Bywaters,[13] in his work on the anatomical changes in the liver following crush injury and various types of trauma, paid special attention to mitotic activity in these livers. He saw numerous mitotic figures in the livers of 7 of 9 patients dying 6 days or more after severe injury. He pointed out that the extraordinary increase in mitotic activity is not a reaction to hepatic necrosis, since it was observed without necrosis, but may be a sequel to reparable damage of the liver parenchyma.

A characteristic and very peculiar feature of the mitotic reaction which occurs in livers without pronounced morphologic changes is its restriction to a more or less definite time interval. A definite mitotic wave was observed in the livers of rats subjected to injections of thiourea. Mitotic figures appeared 48 hours after the first injection, the peak was reached after 53 to 56 hours, and the mitotic reaction subsided after 72 hours. The figures declined in number and disappeared

completely even when administration of thiourea was continued. A new mitotic wave could be provoked after a rest period of several days (about 10 days) and a renewed administration of thiourea. The following experiment illustrates this: Rat 550 received 3 daily injections of 0.4 gm. of thiourea; after an interval of 10 days the same treatment was repeated. The rat was killed 53 hours after the first injection of the second series. Mitotic figures in considerable numbers were present in the liver. It follows that (a) there is a certain latent period between the administration of the drug and the mitotic response, (b) the liver loses the ability to react by mitosis and becomes resistant to further administration of thiourea, and (c) the lack of response to the drug is not permanent and the mitotic reaction reappears after a certain rest period.

Our observations on the time limits of the mitotic reaction parallel those of Wilson and Leduc[10] on the livers of mice injected with tissue extracts. These authors described waves of mitosis, the peak appearing between the fourth and seventh day after the injection. Analysis of the reports from Pfuhl,[3] de Walsche,[6] Mayer,[7] and Deane[8] reveals that the mitotic reaction following the administration of staphylococcus toxin, trypaflavine, and trypan blue is similarly subject to time intervals and limited to a definite period.

It appears from the foregoing that the liver possesses remarkable mitotic potentialities which can be awakened under a wide variety of conditions. Not only may loss of parenchyma following removal of parts of the organ, or necrosis due to administration of liver poisons, initiate a mitotic response of the liver, but also many drugs of different nature as well as tissue extracts, not leading to necrobiosis necessitating repair, may evoke mitotic reactions of no less intensity than those following the destruction of parenchyma. This must be taken into consideration for the understanding of the mechanism of the mitotic reaction in the liver in general and of so-called compensatory mitosis in particular. It is conceivable that the mechanism of the mitotic reaction may be the same in livers with or without loss of hepatic tissue.

Several explanations have been suggested for the mechanism of restoration of liver after loss of parenchyma. Increased functional demand on the remaining liver tissue has been proposed as the main causative factor. Some authors believe that increased nutritional supply to the remaining liver cells is the cause of mitosis. Others assume that regeneration is due to architectural changes resulting from distortion of the capillary network and loosening of liver texture. All these explanations are highly hypothetic. The essential cause of the mitotic reaction in the liver occurring after treatment with substances not affecting the

structural integrity of the organ is likewise difficult to determine. Looking for an explanation for this peculiar phenomenon, consideration must first be given to the possibility that the appearance of increased numbers of mitotic figures in an organ could be due to their accumulation as a result of mitotic arrest. The appearance of mitotic figures in the livers of rats treated with thiourea cannot be explained in this way for the following reason: The presence of all phases of mitosis, including telophases and reconstruction phases without preference for one special stage, speaks against the possibility of mitotic arrest, since mitotic figures usually are arrested at one definite phase. It is reasonable, therefore, to assume that not arrest but activation of cellular division is responsible for the appearance of mitotic figures in the livers of rats treated with thiourea.

Initiation of cellular division in the tissues of the body is conceivable in two ways. One is direct stimulation of cell division in a given organ by the substance applied. The other possibility is that the substance administered to the animal primarily produces changes, at times undetectable, in the organ and that the cells respond secondarily with increased multiplication.

The number of substances considered as activators of cellular division is very limited. They are certain tissue extracts and fractions of these extracts, products of fibrin hydrolysis, sulfhydryl groups, nucleic acids, and nucleoproteins. There is no reason to assume that thiourea belongs to the category of substances which possess the ability to activate cell multiplication primarily. The same applies to other substances known to provoke mitosis in the liver without visible damage to the parenchyma (staphylococcus toxin, trypaflavine, and trypan blue). Moreover, it is very doubtful whether mitotic figures can be initiated in the resting tissues of the living body even by factors known as direct stimulants of cell multiplication.

In the light of these considerations we are inclined to assume that the mitotic reaction in the livers of rats treated with thiourea is the consequence of primary changes in the organ produced by the substance applied. This would mean that increased cell multiplication follows not only lesions associated with loss of parenchyma, but also cell changes without demonstrable morphologic abnormalities. The possibility that the mitotic reaction is not dependent upon the amount of hepatic tissue lost or upon the functional demand placed on the remaining tissue has already been indicated by MacMahon[12] and by Schultz, Hall, and Baker.[14]

The appearance, also, of mitotic figures in a wide variety of conditions in man is not surprising in the light of the above findings. It is

conceivable that mitotic reactions in the liver occur as a result of the action of toxic substances, not necessarily exogenous, which reach the liver and damage liver cells without necessarily producing obvious morphologic changes. This might easily escape detection owing to the time limitation of this reaction. Indeed there is circumstantial evidence that multiplication of hepatic cells by mitotic division occurs more often than is generally realized. The presence of hypertrophy and hyperchromatism of nuclei, the increased number of binucleated and multinucleated cells, the enlargement of nuclei suggesting polyploidy (Sulkin,[15] and Ashworth and Reid[16])—all indirect signs of regeneration in normal livers—are very suggestive of preceding mitotic activity.

Summary

Thiourea given to rats in large doses intraperitoneally induces a striking mitotic activity in the liver in the absence of necrobiotic changes in the parenchyma. The mitotic response in the liver caused by thiourea has a specific time limitation; the mitotic figures appear 48 hours after the first injection, the peak is reached 53 to 56 hours, and the mitotic reaction subsides after 72 hours. Mitotic figures of both normal and pathologic configuration were encountered. Scattering of chromosomes was the main alteration. The mitotic reaction, with or without loss of parenchyma, appears to be a consequence of primary changes in the organ produced by the substance applied.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

PLATE 132

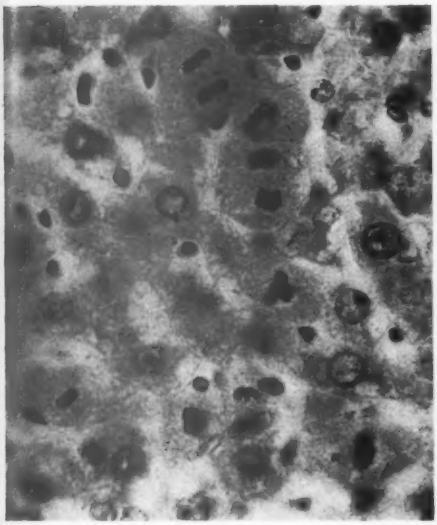
All sections were stained with hematoxylin and eosin.

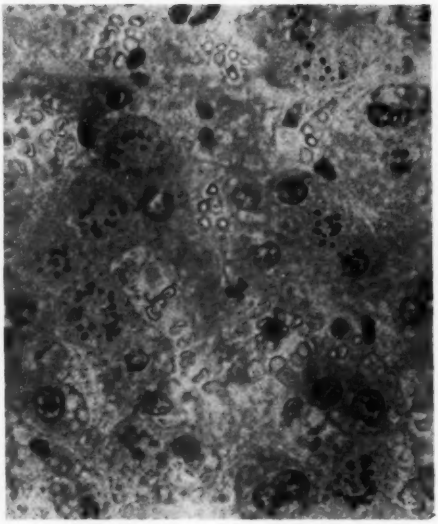
FIG. 1. Rat 376, treated with 3 intraperitoneal injections of 0.4 gm. of thiourea on 3 consecutive days. Normal mitotic figures in liver cells. × 550.

FIG. 2. Rat 372, treated with 3 intraperitoneal injections of 0.4 gm. of thiourea on 3 consecutive days. Mitotic figures with scattered chromosomes in liver cells. × 550.

FIG. 3. Rat 386, treated with 3 intraperitoneal injections of 0.4 gm. of thiourea on 3 consecutive days. Liver cell showing scattering of chromosomes. The chromosomes are of different sizes. × 1600.

FIG. 4. Rat 386. Scattered chromosomes in liver cell, some appearing like diplococci. × 1600.





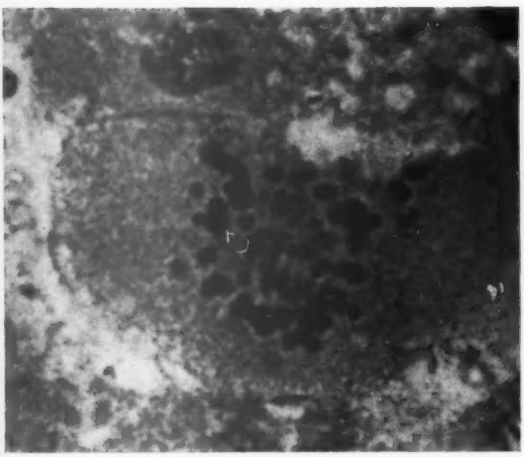


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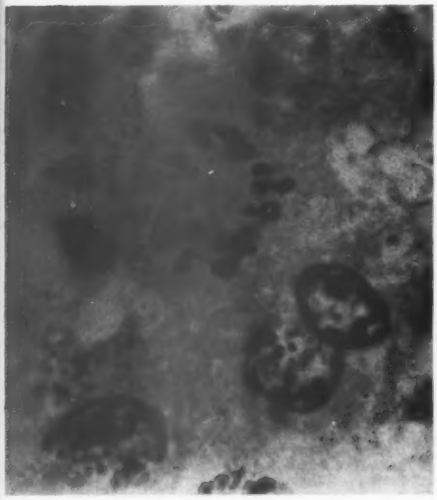
PLATE 133

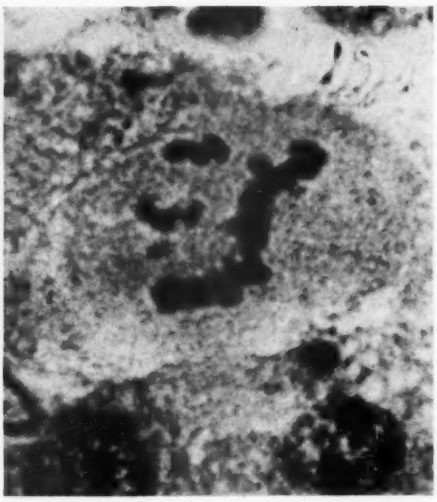
FIG. 5. Rat 386. Chromosomes in liver cell forming a sphere. × 1600.

FIG. 6. Rat 386. Liver cell in mitosis. Most of the chromosomes are arranged in the metaphase plate, while a few are scattered in the vicinity. × 1600.

FIG. 7. Rat 386. Liver cell showing beginning formation of micronuclei. × 1600.

FIG. 8. Rat 386. Liver cell with megakaryocyte-like nucleus. × 1600.





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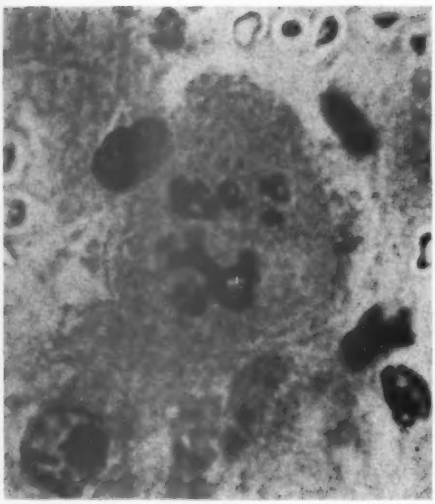

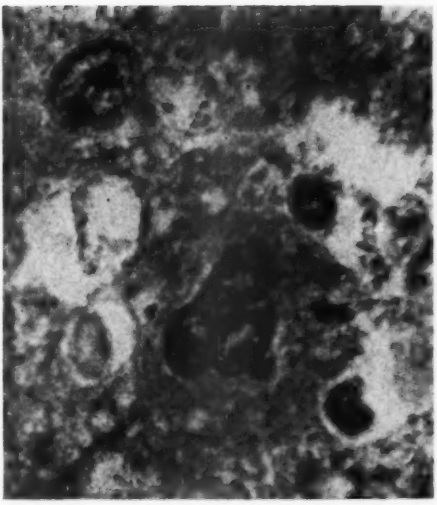

8

Rachmilewitz, Rosin, and Doljanski Mitotic Reaction Induced by Thiourea

THE IMPORTANCE OF METHIONINE AND CHOLINE IN THE ARREST OF DIETARY CIRRHOSIS OF THE LIVER IN THE RAT*

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The relationship of rations high in fat but poor in protein and choline to the production of hepatic fibrosis in rats, dogs, and rabbits has been demonstrated repeatedly.[1] Furthermore, except for the presence of ceroid,[2] the fatty changes, cellular degenerations, and scarring resemble those of human portal cirrhosis. Several investigators[3-7] have established the importance of a deficient intake of lipotropic factors, especially methionine and choline, in the production of hepatic fibrosis and its accentuation by cystine. Nevertheless, there is no clear evidence of the precise rôle of these dietary essentials in cirrhosis and little study has been made of the corrective influence of these substances on an already developing cirrhosis.

Much of the confusion regarding the effects of diets upon the disease process exists because the problem has been investigated using natural foods with which it is difficult to separate quantitatively the dietary factors which prevent or ameliorate cirrhosis. Since it is now feasible to use synthetic diets adequate in all known dietary essentials with dietary nitrogen supplied by 16 crystalline amino acids,[8] it is possible to study more adequately those elements which might be intimately associated with the progression and regression of dietary hepatic fibrosis.

The present experiments were designed to study the effects of varying quantities of methionine, choline, and cystine upon the progress of an already developing cirrhosis. A preliminary experiment was performed which demonstrated that the feeding of a ration modeled after that of György and Goldblatt[3] resulted in early fibrotic changes within 70 days and in well developed cirrhosis consistently by 111 days. Furthermore, treatment with an amino acid ration containing relatively high levels of methionine appeared to arrest the hepatic fibrosis. Therefore a more extensive investigation was undertaken, the results of which are presented in this communication.

* The research which this paper reports was undertaken in cooperation with the Navy Department Office of Naval Research. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or indorsement of the War Department.

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Experimental Procedure

The rats were fed a high fat, low protein diet for 70 days in order to produce hepatic damage. They were then offered rations containing amino acid mixtures with various quantities of methionine, cystine, and choline for an additional 32 days.

Table I

Composition of Basal Ration in Amounts Offered Per Rat Per Day

Diet constituents	Units	Amount
Casein (vit. test)	gm.	1.0
Beet sugar	gm.	6.4
Heated lard	gm.	2.0
Unheated lard	gm.	0.0
O and M salt mixture*	gm.	0.4
Cod liver oil	gm.	0.2
Calcium pantothenate	gamma	125.0
Pyridoxine HCl	gamma	25.0
Riboflavin	gamma	31.0
Thiamine HCl	gamma	25.0
Choline chloride	mg.	0.0
Calories	cal.	49.4
Amount fed per day	gm.	10.0

* Hawk and Oser's modification of the Osborne and Mendel salt mixture plus 1 gm. each of copper sulfate and zinc chloride added to the trace elements.

Diets and Methods of Feeding. The composition of the basal diet is given in Table I as quantities offered per rat per day. Containing about 8.2 per cent protein (N × 6.25), this ration provided almost 5 calories per gm. Deficiency of alpha-tocopherol has been found to play a rôle in the production of dietary liver disease,[9] and this substance is said to be destroyed when unsaturated fats become rancid.[10] Therefore, to facilitate the production of hepatic fibrosis, commercial rendered lard was heated at 110° to 120° C. for 5 to 6 hours and oxygenated with a stream of air before being added to the basal ration. Estimations of average diet consumption per rat were made by daily weighing of the uneaten ration and weekly measuring of the wasted food.

Animals. Sixty-three male Sprague-Dawley rats, approximately 2 months of age, were used as subjects. Initial weights averaged 137 gm. and ranged from 89 to 197 gm. The animals were grouped according to weight in order to reduce excessive competition for food and 7 or 8 were kept in each large wire-bottomed cage. Fifty rats received the basal ration for 70 days. The remainder were fed modified basal rations which will be described later.

Method of Study of Effects of Methionine, Cystine, and Choline

Table II describes the rations containing the amino acid mixtures, and Table III gives the percentage composition of the basal mixture of

14 sulfur-free crystalline amino acids. This mixture was incorporated into the diets at such a level as to provide these 14 amino acids in the quantities present in the casein contained in the basal ration[11] except for minor modifications previously described.[8] To aliquots of this mixture were added the desired amounts of methionine and cystine and all

TABLE II

Composition of Amino Acid Rations in Amounts Offered Per Rat Per Day

Diet constituents	Units	Diet group numbers							
		1	2	3	4	5	6	7	8
dl Methionine	mg.	100.0	20.0	100.0	20.0	100.0	20.0	100.0	20.0
l (−) Cystine	mg.	80.0	80.0	3.1	3.1	80.0	80.0	3.1	3.1
Choline chloride	mg.	50.0	50.0	50.0	50.0	0.0	0.0	0.0	0.0
Amino acid basal mixture	mg.	970.8	970.8	970.8	970.8	970.8	970.8	970.8	970.8
Other ingredients*	gm.	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Amount fed per day	gm.	10.2	10.1	10.1	10.0	10.2	10.1	10.1	10.0
Per cent of nitrogen		1.28	1.24	1.25	1.18	1.26	1.25	1.16	1.09

* Other ingredients = the quantities of dietary constituents in the basal ration excepting the casein.

TABLE III

The Percentage Composition of Basal Mixture of Sulfur-Free Amino Acids

Amino acid and form		Per cent
dl	Alanine	4.90
l (+)	Arginine HCl	4.34
dl	Aspartic acid	5.52
l (+)	Glutamic acid	20.76
	Glycine	0.44
l (+)	Histidine $HCl \cdot H_2O$	2.96
dl	Isoleucine	11.38
l (+)	Leucine	10.59
l (+)	Lysine $HCl \cdot H_2O$	8.29
dl	Phenylalanine	4.55
dl	Threonine	6.83
dl	Tryptophane	1.58
l (−)	Tyrosine	5.60
dl	Valine	12.26
	Total	100.00

dietary ingredients were blended in a mechanical mixer for 20 minutes. The quantities of methionine and choline were chosen to provide the animals with approximately those amounts reported by György and Goldblatt[8] as adequate to prevent dietary cirrhosis. The assumption was made that the rats would eat the same quantity of ration in the treatment period as in the preliminary experiment. The low methionine diet contained enough of this amino acid to prevent the loss of appetite associated with severe methionine deficiency. The ration containing the largest amount of cystine was calculated to furnish the same quantity

of sulfur as that with the highest level of methionine. The low cystine level provided the quantity present in 1 gm. of casein, a protein low in cystine.[11]

A factorial design was adopted for the experiment.[12] Thus with three variables, methionine, choline, and cystine, at two levels each, eight diet combinations were required. Eight groups of 5 rats each were selected at random from the large group fed the preparatory basal ration for 70 days. They were placed in individual cages and fed the amino acid rations described in Tables II and III for the 32 days of the treatment period. One group of 5 animals was sacrificed immediately prior to initiation of the treatment period to provide information concerning the pathologic changes present at that time. Another group also kept in individual cages continued to receive the basal ration during the final 32-day period. At the end of the experiment, 102 days after the start of the basal ration, all surviving rats were sacrificed and the livers weighed after exsanguination.

Weight, Hemoglobin, and Serum Protein Determinations

The rats were weighed at weekly intervals after a 12-hour fast, and twice weekly during the last 32 days. Hemoglobin and serum protein concentrations were determined by methods previously described[13] on blood obtained by cardiac puncture at the time of sacrificing.

Histologic Findings

Tissues obtained at necropsy were fixed in 10 per cent formol-saline solution. Frozen sections of the liver, kidney, and heart were stained for fat with oil red-O. Celloidin-imbedded, hematoxylin and eosin preparations were made from blocks cut through the entire right and left lobes of the liver, as well as of the lung, heart, thymus, spleen, pancreas, kidney, adrenal, and testis. Selected liver sections were treated with Cooper's* modification of the Ziehl-Neelsen stain to demonstrate the presence of acid-fast ceroid, and with Mallory's connective tissue stain to permit more adequate estimation of the degree of hepatic fibrosis.

Experimental Results

The basal ration permitted slow growth for about 50 days, despite a small weight loss during the first 10 weeks. Diet consumption then decreased and the animals lost weight. They appeared emaciated, with greasy, scant fur, and yellow, crusted ears and mouths. At the end of 70 days their average weight was only 2 per cent above their initial

* Gay, D. M. Cooper's modification of the Ziehl-Neelsen staining method as applied to tubercle bacilli in tissue. *J. Lab. & Clin. Med.*, 1932, **17**, 1131–1132.

weight. The 5 animals sacrificed at this time had large, pale, tan, smooth livers with an average weight of 12.5 gm. as compared with the normal of about 6 gm. for rats of comparable body weight. Histologic examination revealed a severe, small droplet, fatty change, slightly less marked about the central veins, and many large fat globules in the peripheral areas. The enlarged lobules were composed of cells with granular, pink cytoplasm in the central zones, swollen reticulated midzonal cells, and vacuolated peripheral cells. In 3 of the 5 rats there were focal accumulations of lymphocytes and fibroblasts between cord cells and around blood vessels. In some places these formed bands connecting a few central veins with each other and with portal triads (Figs. 1 and 2). Small collections of macrophages and isolated parenchymal cells were filled with foamy yellow, acid-fast material. These were often most prominent at the sites of connective tissue proliferation. Some cells were undergoing degeneration with hyaline cytoplasm and pyknotic nuclei, but no areas of hemorrhagic necrosis were noted. The other organs examined were grossly and microscopically normal.

The concentrations of blood constituents in the animals sacrificed at 70 days were all below normal, the decrement being most marked in the hemoglobin concentration.

The treatment rations containing amino acids had the following combinations of methionine, cystine, and choline:

Diet 1.....high methionine, high cystine, with choline
Diet 2.....low methionine, high cystine, with choline
Diet 3.....high methionine, low cystine, with choline
Diet 4.....low methionine, low cystine, with choline
Diet 5.....high methionine, high cystine, without choline
Diet 6.....low methionine, high cystine, without choline
Diet 7.....high methionine, low cystine, without choline
Diet 8.....low methionine, low cystine, without choline

Table IV summarizes the data on weight changes and the average daily consumption of methionine, cystine, and choline. During the first week of the amino acid ration food consumption was poor and the animals lost 10 to 18 per cent of their initial body weight, while the rats continuing to receive the basal ration lost only 4 per cent, and ate as much as before. In the subsequent 25 days the animals on the diets containing the high level of methionine or cystine plus choline (diets 1, 2, 3, and 5) gained steadily. Those on diet 7 only maintained their weight. Despite the gains, only a few rats recovered from the loss of the first week. Rats on these rations grew new fur, and the mouth and ear crusting diminished. Rats on the remaining three amino acid rations, all of which were low in methionine, lost weight, despite a diet

consumption about equal with those which gained weight. Hemoglobin levels were not greatly different after this final 32-day period, but the serum protein levels when compared with those of rats killed after the 70-day period, were significantly higher in animals on diets 1 and 3. No animals died in the group receiving diet 3, but 1 to 3 animals from each of the other groups died (Table V).

TABLE IV

Average Weight Changes, and Consumption of Methionine, Cystine, and Choline

Diet	No. of days on diet	No. of rats*	Initial weight	70 day weight change†	Weight change on treatment†		Mean daily diet consumption (%)
					7 Days	32 Days	
			gm.				
Basal	70	5	134	+10			87
1	32‡	3	150	+ 4	−14	− 7	55
2	32‡	4	134	+ 5	−16	− 9	56
3	32‡	5	144	− 1	−17	−11	57
4	32‡	4	156	+ 7	−14	−17	66
5	32‡	4	141	+11	−16	− 6	66
6	32‡	2	122	+ 5	−18	−20	53
7	32‡	4	166	− 1	−10	− 9	69
8	32‡	3	144	+ 3	−13	−27	54
Basal	102	2	131	+12	− 4	−13	61

* Animals sacrificed after 70 and 102 days.

† Percentage weight change calculated as total grams gained or lost per 100 grams of original body weight.

‡ Days on amino acid rations after 70 days on the basal ration.

After the final 32-day period the livers of rats receiving rations low in methionine and lacking choline (diets 6 and 8) as well as those fed the basal ration were large, yellow-tan, cut with increased resistance, and had fine granular surfaces with wrinkled areas on the posterior and inferior aspects. When either choline or methionine was provided in large amounts (diets 2, 4, and 7), scarring was less marked. Reddish tan, smooth livers were present in the rats fed diets 1, 3, and 5 with high methionine plus choline or high cystine. Despite the differences in consistency and color, all livers were unusually large with no significant weight differences in the small groups of animals studied (Table V).

The changes observed in the other organs examined were usually correlated with the degree of hepatic fibrosis. Gross and histologic examination revealed lymphoid depletion and atrophy of the spleen, decreased spermatogenesis, and occasional foci of pneumonia in animals with severe cirrhosis. No significant renal changes were apparent.

The histologic changes in the livers were graded. Cirrhosis was evaluated from 0 to 4 (no scars present to severe distortion of architecture by many small and some large bands of connective tissue). The degree of large droplet fatty change was graded from 0 to 5 (no or rare isolated globules to uniform, severe involvement), and the small droplet altera-

TABLE V

Data on Number of Deaths, Liver Weights, and Degrees of Pathologic Changes

Diet	No. of days on diet	No. of rats	No. of rats dying	Average liver weight*	Number of rats with each degree of histologic change															
					Hepatic fibrosis					Small droplet fat					Large droplet fat					
					0	1+	2+	3+	4+	0	1+	2+	3+	4+	0	1+	2+	3+	4+	5+
				gm.																
B–A	70	5		12.51	2	3							1	4		1	2	1	1	
B–B	70	2		14.15	1	1						1	1							2
B–C	70	3		11.95	3								3		2	1				
1	32†	5	2	11.31	1	2	1‡	1‡			1‡	1‡		3		2	1	1‡	1‡	
2	32†	5	1	11.46	1	1	3‡					1‡	4			2	1	1‡	1	
3	32†	5	0	11.62		4	1						3	2	1	2	2			
4	32†	5	1	9.97		2		2‡	1		2‡	1	2			1		4‡		
5	32†	5	1	11.23		2	2‡	1				1‡	2	2		4‡	1			
6	32†	5	3	10.23			2§	3‡			2§	3‡						2§	2	1
7	32†	5	1	12.49		2	1	1‡	1		1‡	2	2			1	3‡	1		
8	32†	5	2	12.04		2‡	1‡		2		3‡	2‡				1‡			3‡	1
B–A	102	5	3	14.86			2‡		3§		2§	3‡							5§	
B–B	102	4	1	12.31		3‡		1			1	1	2‡					1‡	3	
B–C	102	4	0	12.37	2	1	1						1	3	3	1				

* Average liver weights of animals not dying spontaneously.

† Days on amino acid ration after 70 days on the basal ration (diet B–A).

‡ Includes one animal dying during the last 32 days of the experiment.

§ Includes 2 or more animals dying during the last 32 days of the experiment.

tion was estimated from 0 to 4 (no stainable fat to small droplets present in almost all cells). Three observers graded the sections without knowledge of the animals' dietary history and were in essential agreement in all cases. These findings are summarized in Table V along with the number of deaths per group and the average liver weights of surviving rats.

The factorial design used in these experiments is capable of answering with a considerable degree of precision several questions.[12] These are as follows: Does methionine, cystine, or choline alone in the quantities offered observably influence the course of the hepatic damage induced by the preliminary treatment? Furthermore, do any of the combinations of two of these ingredients have any effect over and above that which each may have alone; that is, is there any synergistic or antagonistic action? Finally, is the combination of all three of the ingredients any more or any less effective than when each is administered alone or in paired combinations with the others? In Table VI we have

TABLE VI

The Effect of Methionine, Cystine, and Choline Alone or in Their Several Combinations Upon Hepatic Changes Induced by Dietary Means

Treatment	Effect on cirrhosis	Effect on large droplet fatty change	Effect on small droplet fatty change
Methionine	+ (p <.02 >.01)	+ (p <.001)	− (p <.001)
Cystine	0	0	0
Choline	+ (p <.05 >.02)	+ (p <.001)	− (p <.02 >.01)
Methionine, choline	0	+ (p <.001)	0
Methionine, cystine	0	0	0
Choline, cystine	0	+ (p <.02 >.01)	0
Methionine, cystine, choline	0	+ (p <0.01 >.001)	0

summarized the results of the analysis of the data with respect to the various treatments upon the degree of cirrhosis, the large droplet, and the small droplet fatty changes. In the table a plus (+) sign indicates that a statistically significant ameliorating effect was obtained from a particular treatment combination. Zero (0) indicates no significant effect, and a minus (—) sign indicates an increase in the degree of pathologic change observed. The "p" values given with each of the plus or minus signs indicates the order of significance of the observed effect.

It is apparent from Table VI that methionine and choline alone each had an ameliorating effect upon the cirrhosis. Cystine and the various combinations of the three therapeutic substances had no effect upon the cirrhosis. The influence of methionine and choline upon the small drop-

let fatty change was the reverse of the effect upon cirrhosis. The other therapeutic combinations had no effect upon the small droplet fatty change. Methionine and choline caused improvement in the large droplet alteration. In addition the combination of methionine and choline, choline and cystine, and methionine, cystine and choline produced further improvement beyond that of the single ingredients.

The following is a brief description of the histologic findings. Distortion of hepatic architecture was moderate to severe in animals fed low methionine rations without choline (diets 6 and 8) and the basal ration. Nodules of hepatic parenchyma demarcated by bands of young fibroblasts and ceroid-filled macrophages (Fig. 3) contained degenerating cells. In other areas the cytoplasm was foamy or severely vacuolated (Fig. 4). The nuclei were large, abnormal, and often had several nucleoli. Foci of closely spaced, newly formed, small bile ducts occurred near the scars. Small zones of hepatic necrosis were found only occasionally. Slightly less distortion of architecture by small irregular fibroblastic scars, often focal, was present in the livers of rats fed diets 2, 4, and 7 which contained either choline or methionine in the larger amounts. The small droplet fatty change was more marked, while the large globules were restricted to cells along lobular margins or near fibrous scars. Cells in the centers of some nodules contained granular, often basophilic, cytoplasm and had actively dividing nuclei, perhaps indicating some regeneration. Most of the livers of animals that received rations high in methionine with either choline or the high level of cystine (diets 1, 3, and 5) exhibited only isolated connective tissue bands of about the extent present in rats after 70 days on the preparatory ration (Fig. 5). The cells were swollen and reticulated with a moderately severe small droplet fatty change and occasional large globules (Fig. 6). The nuclei and hepatic cords were most nearly normal in these livers, except when modified by parenchymal regeneration. Regeneration was most prominent in groups receiving diet 3.

In addition to amino acid therapy the influence of unheated lard as opposed to heated lard in the basal ration, and the effects of the addition of choline to the basal ration were observed. To do this an additional group of 6 rats was fed a modified basal diet in which unheated lard was substituted for the heated aerated fat. A second group of 7 rats received the basal diet supplemented with 2.5 mg. of choline chloride per gm. of diet. Two rats from the first of the groups were sacrificed at the end of 70 days along with 3 from the choline-supplemented group. The remainder were sacrificed at 102 days. The animals receiving the unheated lard showed liver changes almost identical with those receiving the unmodified basal ration. The only appreciable difference was

that large droplet fatty change appeared more prominent at 70 days and cirrhosis slightly less marked at 102 days. The group receiving choline supplement showed no hepatic fibrosis at 70 days but did exhibit a rather severe generalized small droplet fatty change. At 102 days there was still marked small droplet fatty change with only occasional large droplets. Only rare accumulations of fibroblasts and ceroid were apparent.

Discussion

The arrest or delay of dietary cirrhosis with improvement in the parenchymal changes by the consumption of relatively large amounts of methionine and choline may be related to their lipotropic properties. Both substances are lipotropic under certain conditions,[14,15] perhaps requiring the conversion of methionine to choline *in vivo*.[16] By combining with fatty acids to form phospholipids, choline facilitates the transport of fats from the liver.[17] The alterations in histologically demonstrable fat suggest that lipotropic agents may initiate a splitting of the large droplets to smaller ones before withdrawing the fat almost completely from the liver cells, and that when a deficiency of lipotropes exists, the small droplets can coalesce to form large globules. Choline does not seem to be effective in preventing or reducing small droplet fatty change. Accumulation of this type occurred in rats fed a low protein, low fat diet with presumably adequate choline intake[18] and in animals fed the high fat basal ration with 25 mg. of choline chloride per day. Furthermore, choline therapy in these experiments diminished cirrhosis and large droplet fatty change but increased the small droplet change. Dietary cirrhosis may depend partially on the effect of accumulation of fat in the hepatic parenchyma.[19] In our experiment a high correlation between the degree of hepatic fibrosis and the degree of large droplet fatty change was noted (correlation coefficient = 0.89). Therefore, the lipotropic action of methionine and choline, by removing the large droplets, might, in part, explain their ability to arrest hepatic fibrosis. However, this formulation does not solve the problem, since fatty infiltration can occur without subsequent fibrosis,[19] cirrhosis can, under certain conditions, develop without accumulation of fat,[20] and slight scarring did appear in rats fed the choline-supplemented diet.

Previous investigations have shown that cystine supplements to low casein, high fat diets aggravate, while additions of cystine plus choline prevent, dietary cirrhosis.[21,3,5] Under the conditions of our experiment, hepatic fibrosis was not increased by the high level of cystine. That the combination of cystine and choline was not effective in arresting cirrhosis, although it did reduce the large droplet fatty change, might be due to the inability of the already diseased liver to utilize cystine and

choline, or it is possible that they were not available in optimal proportions.

Under the circumstances of the experiment mortality rates on the various diets were not significantly different.

The amount of acid-fast material, presumably ceroid, in macrophages and parenchymal cells was proportional to the degree of hepatic fibrosis. However, it has been found that ceroid is not an essential antecedent to dietary cirrhosis.[22] Our animals received cod liver oil, which is reported to contribute to the formation of ceroid.[23] Alpha-tocopherol deficiency may also be a factor in its production,[24] and it was observed that slightly less cirrhosis and ceroid formation occurred in rats fed the unheated lard ration for 102 days than in those fed heated oxygenated lard in the basal diet. The appearance of the animals after 70 days on the basal diet resembled that described by Victor and Pappenheimer[24] and attributed by them to an alpha-tocopherol deficiency. These changes were ameliorated by feeding diets containing large amounts of methionine and choline for 32 days.

Recent studies have demonstrated improvement of dietary cirrhosis when choline, methionine, and casein supplements were fed rats after a preparatory period on a low protein, high fat ration. Lowry and coworkers[25,26] found that even prolonged treatment with choline had no apparent effect on the fibrous tissue, but regenerative changes and arrest of hepatic fibrosis occurred. In our relatively short treatment period of 32 days no resolution of fibrosis already present could be proved. However, the finding of apparently regenerating nodules and more normal cord cells in livers of rats fed the choline supplemented rations, especially with high methionine levels, suggested that these dietary essentials have some curative effect on the parenchymal changes. György[27] treated rats with high methionine and high casein diets for 150 days after a 150-day preparatory period and concluded that even severe fibrosis might disappear if proper dietary measures were introduced and continued for a sufficient length of time.

Treatment of human cirrhosis with lipotropic factors and high protein diets has been found to produce parenchymal changes similar to those observed in our experiment. Studies with serial liver punch biopsies have revealed disappearance of fatty infiltration and more normal hepatic cord cells.[28-30] Therefore, the conclusion is warranted that treatment of cirrhosis with methionine and choline, whether as supplements or in high protein diets, has definite value in both the rat and man.

Further investigations might contribute to the understanding of the mechanisms involved in the arrest of experimental dietary cirrhosis

that are not evident from the present study. Feeding larger amounts of amino acid nitrogen than that in the basal rations and the synthetic diets used here, varying the level of fat in the treatment rations, adding alpha-tocopherol to the diets, and continuing the experimental period for a longer time might reveal further significant changes.

Summary and Conclusions

An experiment with young albino rats is described in which it was possible for the first time quantitatively to evaluate the influence of methionine and choline on the progression of dietary cirrhosis. After a preparatory period of 70 days on a low protein, high fat ration, the animals were offered diets containing crystalline amino acid mixtures in place of casein with various quantities of methionine, cystine, and choline for 32 days. When the rats ate 55 to 69 mg. of methionine or 27 to 33 mg. of choline chloride per day, cirrhosis was absent or much less marked than when they consumed about 11 mg. of methionine and no choline. No accentuation of hepatic fibrosis was noted that could be attributed to high levels of cystine, and the combination of cystine plus choline was not as effective in arresting the scarring as were methionine and choline. The influence of methionine and choline on the cirrhosis was closely correlated with the ability of these dietary essentials to alter the large droplet fatty change present in rats after 70 days on the basal ration. These substances, perhaps through their lipotropic properties, caused a disappearance of the large globules, and this improvement in the structure of the parenchymal cells may, in part, have delayed the progression of the hepatic fibrosis. The similarity in the histologic alterations observed in these rats to the changes produced by treating cases of human cirrhosis with lipotropes suggests that methionine and choline have a definite value in the treatment of this disease.

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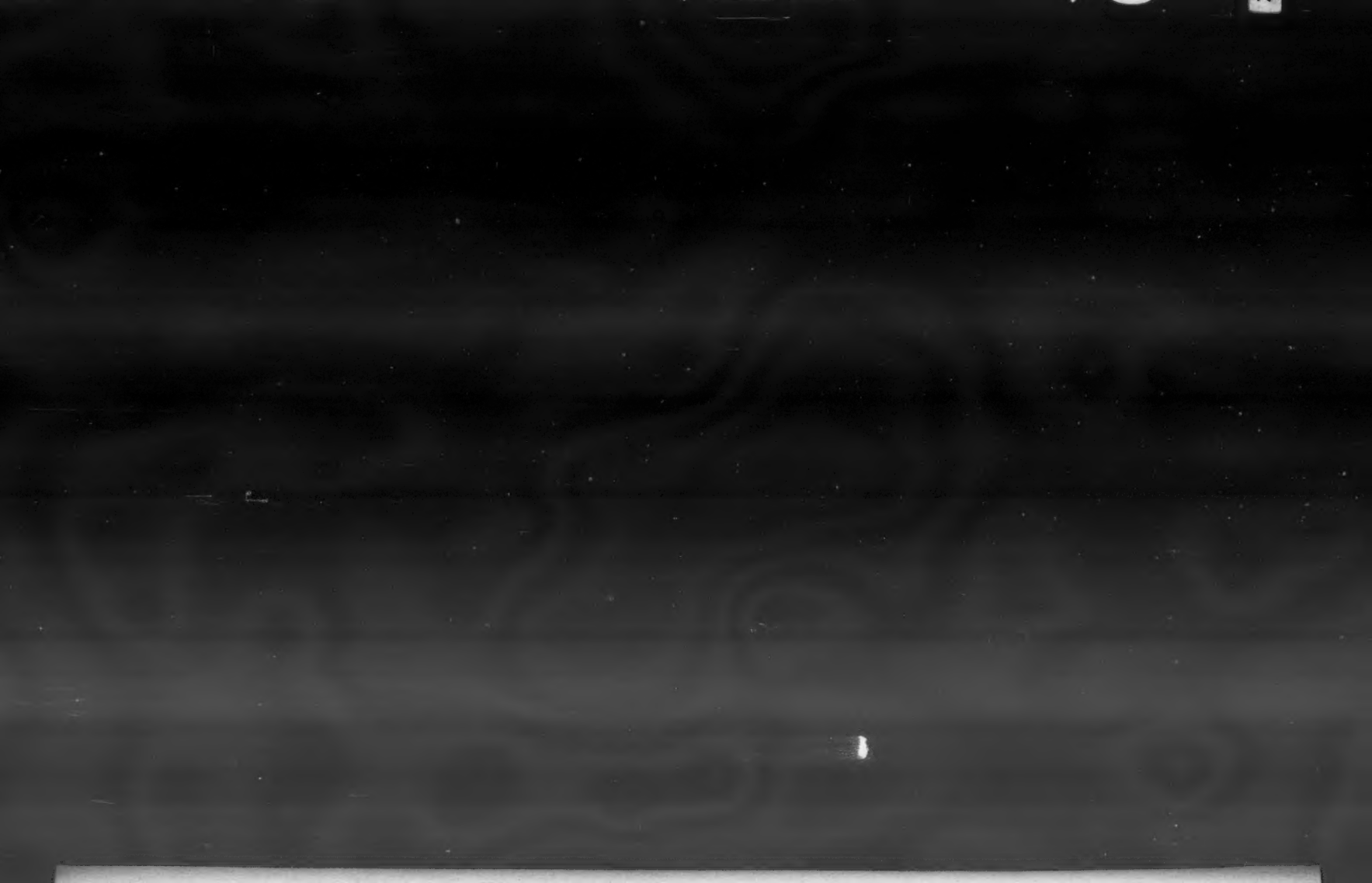
DESCRIPTION OF PLATES

PLATE 134

FIG. 1. Fibrous bands around portal triad and lobule, and between vessels in the liver of a rat fed the basal ration for 70 days. Mallory's connective tissue stain. × 145.

FIG. 2. Macrophages with ceroid in center with fibroblasts and lymphocytes in the parenchyma. Moderately severe large droplet fatty change. Same liver as in Figure 1. Hematoxylin and eosin stain. × 145.

FIG. 3. Severe hepatic fibrosis with distortion of architecture in the liver of a rat fed the low methionine, low cystine diet without choline (diet 8) for 32 days after 70 days on the basal ration. Mallory's connective tissue stain. × 145.



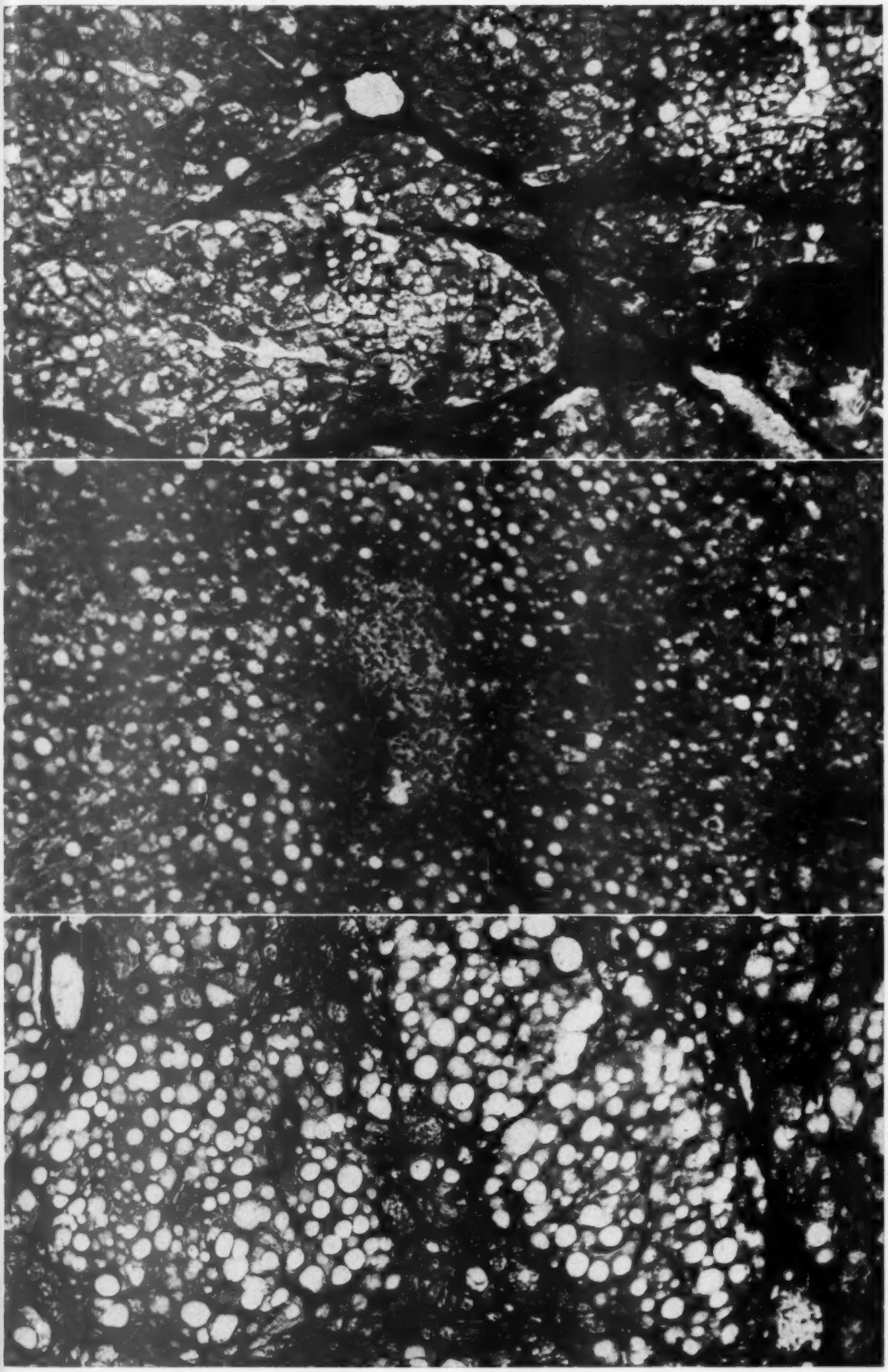
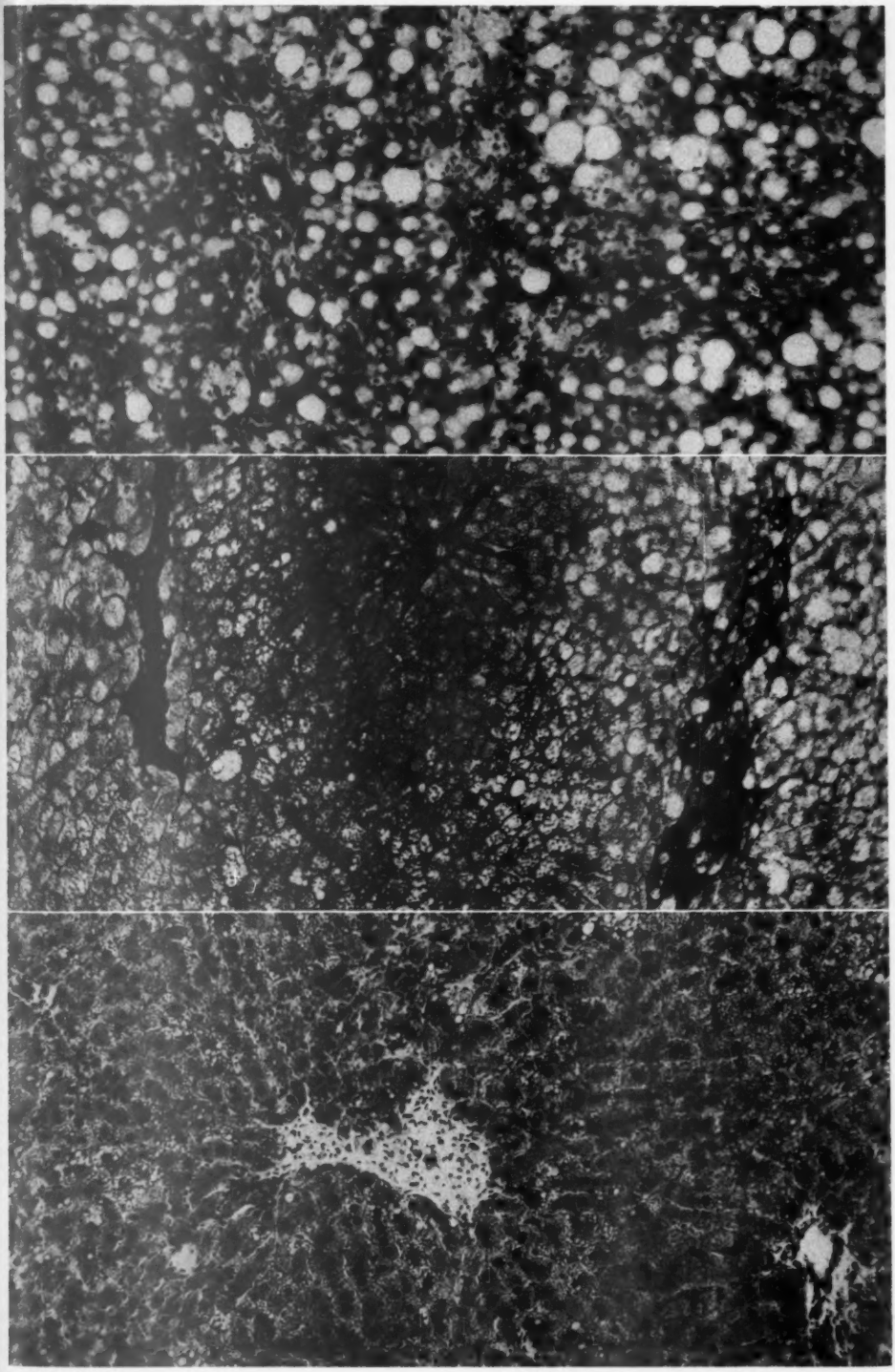


PLATE 135

FIG. 4. Severe large droplet fatty change, bands of fibroblasts, ceroid pigment, and degenerating hepatic cells. Same liver as in Figure 3. Hematoxylin and eosin stain. × 145.

FIG. 5. Minimal perilobular fibrosis in the liver of a rat fed the high methionine, low cystine diet with choline (diet 3) for 32 days after 70 days on the basal ration. Mallory's connective tissue stain. × 145.

FIG. 6. Orderly hepatic cords with a severe small droplet fatty change and rare large droplets. Fibrous scar with ceroid. Same liver as in Figure 5. Hematoxylin and eosin stain. × 145.



Jaffé, Wissler, and Benditt Methionine and Choline in Dietary Cirrhosis

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